Nephroprotective effect of Beekh Kasni (Roots of Cichorium intybus) in the form of methanolic and aqueous extract in Gentamycin induced rat models

Roohi Zaman, Anzar Alam, MA Jafri, G Sofi and Ghufran Ahmad

Abstract
Background: Unani System of Medicine (USM) possesses very effective and safe nephroprotective drugs which are useful in renal disorders for a long time by renowned Unani Physicians. Beekh Kasni (roots of Cichorium intybus) belongs to the family of asteraceae, which is one such type of drug widely used as a diuretic, nephroprotective and tonic to kidneys. It is also used in kidney diseases of diverse etiology, having different pathological (su-e-mizaj, su-e-tarkeeb or tafarruq-e-ittesal) and symptomatic presentations. The drug Beekh Kasni has not been scientifically studied; therefore it is important to evaluate this clinically important drug scientifically. In the present study, an effort has been made to evaluate the effect of the aqueous and ethanolic extract of Beekh Kasni on Gentamicin induced nephrotoxicity in rats. The test drug was studied for nephroprotective activity.

Material Methods: Nephroprotective activity of the test drug was evaluated in wistar rats. Animals were allocated into 6 groups of 6 animals in each. Group I served as plain control and group II served as negative control in which toxicity was induced by using Gentamicin (80 mg/kg IM once daily for 12 days). Third group served as concurrent test group A was administered with ethanolic extract of Beekh Kasni (3.4 mg/kg orally once daily for 12 days along with Gentamicin 80 mg/kg IM). Fourth group served as concurrent test group B was administered with aqueous extract of Beekh Kasni (3.4 mg/kg orally once daily for 12 days). Fifth and Sixth group served as pretreated groups were initially administered ethanolic and aqueous extract of Beekh Kasni 3.4mg/kg respectively orally once daily for 15 days and subsequently Gentamicin 90mg/kg IM once daily administered for the next 12 days. On the 13th day the animals were anaesthetized using phenobarbitone at the dose of dose 50mg/kg, blood was collected by cardiac puncture for the estimation of serum creatinine and serum urea (Bayers) using auto analyzer. Nephroprotective activity was further confirmed by findings of histopathological examination of the kidney tissues where the structural integrity was brought to near normal level in both concurrent and pretreated group of animals.

Conclusion: The test drug Beekh Kasni possesses a significantly high level of nephroprotective effect and the findings validated the Unani description of the test drug.

Keywords: Nephroprotection, Kidney function markers, Zof-e-Kulliya, Beekh Kasni

Introduction
Kidney is the chief excretory organ responsible to excrete the waste, undesirable and toxic substances out of the body. It always remains in direct contact with substances of aversive nature which make it susceptible to toxicity and injury. Many drugs which are used in various ailments are liable to produce nephrotoxicity. Kidney is also taxed with extra demand for work such as in BP, CHF, oedema and diabetes, in order to maintain the homoostasis kidney works excessively, the increased work load over a long period of time causes degenerative changes [1].

Unani System of Medicine (USM) possesses very effective and safe nephroprotective drugs which are useful in renal disorders. The physicians of Unani Medicine are using such drugs effectively since centuries for the treatment of renal disorders with a good recovery rate. Beekh Kasni (roots of Cichorium intybus) (Fig. 1) is one of the drug which is widely used as a diuretic, nephroprotective and tonic to kidneys therefore used in kidney diseases of diverse aetiology, having different pathological and symptomatic presentations [2,4].

Despite being extensively used in clinical practice in USM, the drug Beekh Kasni has not been studied, therefore it is substantial to subject this clinically important drug for scientific evaluation. In the present study, an effort has been made to evaluate the effect of the aqueous and ethanolic extract of Beekh Kasni on Gentamicin induced nephrotoxicity in rats.
Material and methods

Roots of Kasni were collected from the herbal garden of National Institute of Unani Medicine and identified by a botanist, Prof. Najma Bano. HoD Dept. Of Botany, Al-Ameen College, Bangalore. After proper identification, drug was dried in shade and ground with mechanized grinder to prepare a coarse powder.

Aqueous and Methanolic extracts of Beekh Kasni were prepared using soxhlet apparatus and the yield was found to be 24.94% and 24.52% respectively. The doses for both aqueous and methanolic extract of Beekh Kasni were calculated according to Fredrech et al, multiplying by the conversion factor of 7 in rats [5].

The required quantity of the extract was administered to the animals orally by feeding canula and the dose of standard drug Gentamicin was used as 80 mg/kg intramuscularly. The study was approved by the IAEC of NIUM, Bangalore, India, registered under CPCSEA, India (Reg. No 953/c/06/CPCSEA).

Experimental Animals

Adult Albino rats (200-250 gm) of either sex were used for this experiment. The animals were given standard pellet diet and given tap water ad libitum. The experiments were performed in quiet room. The rats were housed in polypropylene cages and maintained under standard conditions (12 hr light & dark cycles, Temp at 25 ± 3°C and 60-70% humidity).

Nephroprotective activity

The test drug was studied for nephroprotective activity by the method of Anwar et al (1999) [6] Albino rats of either sex were divided into 6 groups of 6 animals each randomly. The first group served as plain control was administered with 30 ml of Normal Saline orally once daily for 12 days. Second group of served as negative control was administered with standard drug Gentamicin (80mg/kg IM once daily for 12 days). Third group served as concurrent test group A was administered with methanolic extract of Beekh Kasni (3.4mg/kg orally once daily for 12 days along with gentamicin 80 mg/kg IM).

Fourth group served as concurrent test group B was administered with aqueous extract of Beekh Kasni (3.4 mg/kg orally once daily for 12 days). Fifth and Sixth group served as pre treated groups were initially administered methanolic and aqueous extract of Beekh Kasni 3.4mg/kg respectively orally once daily for 15 days and subsequently Gentamicin 80mg/kg IM once daily administered for the next 12 days. On the 13th day the animals were anaesthetized using phenobarbitone at the dose 50mg/kg, blood was collected by cardiac puncture for the estimation of serum creatinine and serum urea (Bayers) using auto analyzer.

Histopathological examination

Two animals from each group were sacrificed on the day of blood withdrawal and both the kidneys were dissected, washed with saline and preserved in 10% formaldehyde. Then both kidneys were processed and embedded in paraffin wax and sections were taken using microtome. These sections were stained with haematoxylin and eosin and observed under a computerized light microscope.

Statistical analysis

All results from different pharmacological studies are presented as mean. Data were analyzed using ANOVA non repeated measure with post hoc comparison test. Results were considered significant at P < 0.05.

Results

Serum creatinine and Serum urea were significantly elevated in group II treated with Gentamicin whereas treatment with aqueous and ethanolic extracts of Beekh Kasni was found to protect the rats from such effects of Gentamicin. As shown in Table I the pre treatment groups V and VI produced significant protection against kidney damage caused by Gentamicin as compared to post treatment groups III and IV. It is also observed that the effect of aqueous extract of Beekh Kasni is better than methanolic extract of Beekh Kasni in both pre and post treated groups Fig I&II.

Histopathological Examination

The HPE of kidney of plain control group showed normal glomerular and tubular histology. (Fig.3). Whereas negative control group rats showed glomerular, peritubular, blood vessel congestion with presence of inflammatory cells causing severe necrosis. (Fig.4) Concurrent treatment with the extracts of Beekh Kasni was found to reduce such changes in the kidney histology induced by Gentamicin (Fig. 5 & 6). Pre treatment with the extracts of Beekh Kasni was found to prevent the damage caused by Gentamicin. Kidney histology was found to be normal in pre-treatment group with aqueous extracts of Beekh Kasni as in plain control group (Fig.7 & 8)

Table 1: Effect of aqueous and methanol extracts of Beekh Kasni on renal functions in gentamicin induced toxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Creatinine (Mean ± SEM)</th>
<th>Serum Urea (Mean ± SEM)</th>
<th>&quot;P&quot; Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Plain Control</td>
<td>0.466 ± 0.021</td>
<td>15.5 ± 0.763</td>
<td>-</td>
</tr>
<tr>
<td>Group II Negative Control</td>
<td>2.35 ± 0.399</td>
<td>48.850 ± 2.665</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group III (Methanol extract)</td>
<td>1.566 ± 0.434</td>
<td>15.080 ± 2.006</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Group IV (Aqueous extract)</td>
<td>1.066 ± 0.220</td>
<td>34.146 ± 3.646</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Group V Pretreated with</td>
<td>0.8 ± 0.085</td>
<td>14.373 ± 0.444</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Group VI Pretreated with</td>
<td>0.733 ± 0.130</td>
<td>13.666 ± 0.940</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Discussion

In renal disorders the finding of a substantial rise in serum urea and serum creatinine is nearly always good evidence of a severe reduction in glomerular filtration rate. The concentration of serum urea and creatinine depends upon their rate of production and elimination, if their rate of elimination is via glomerular filtration and their daily production is relatively constant, then a fall in glomerular filtration will cause rise in plasma concentration until a new equilibrium is reached, conversely if glomerular filtration remains constant and the rate of serum urea and serum creatinine increases their plasma concentration will also increase, therefore the serum creatinine and urea level gives sufficient information to assess the renal function. A number of studies on these parameters have been carried out to demonstrate nephroprotective effect in the drugs. Nephroprotection is an emerging area of research particularly in the fields of herbal and traditional systems of medicine. Since, a number of drugs have been shown to produce significant nephroprotection and associated effects. Therefore, these are being considered as the promising source of drugs which can be used in the diseases of kidney. A large
group of patients remain untreated or undertreated because western medicine has its own limitations in offering such drugs which can be successfully used in the management of various diseases. A number of herbal drugs which are extensively used in the management of various diseases irrespective of the system or organ produce adverse drug reactions including nephrotoxicity such as Brinjasif (Achillea millefolium), Beekh Kiber (Capparis spinosa), Bisfaj (Polypodium vulgare), Tukhm Gandana (Allium ascalonicum), Dammul Akhwain (Dracaena cinnabari), Sakbeenaj (Ferula persica), Sumbul teeb (Nardostachys jatamansi), Saleekha (Cinnamonum cassia), Tukhm Khurfa (Portulaca oleracea), Tukhm Hammaz (Ramex vesiculans), Zaravand Taveel (Aristolochia indica) [7, 9, 10, 12, 14]. Unani drug Beekh Kasni which is described to be effective in kidney diseases of diverse aetiology [9, 11, 12], was subjected to testing for nephroprotective activity. It has been reported that Gentamicin accumulates in renal tubules and produces local necrosis therefore renal dysfunction in this case represents the renal tubular damage which is generally manifested by the increase level of urea and creatinine in serum and structural changes on HPE. Thus the test drug by decreasing the level of two important markers of kidney function demonstrates that it possesses significant nephroprotective activity which was further confirmed by findings of HPE where the structural integrity was brought to near normal level. One of the mechanisms proposed in Gentamicin induced nephrotoxicity has been oxidative stress. Since the root of Kasni contains good amount of kaempferol, Quercetin- 3 galactoside and bioflavonoids, which are reported to possess antioxidant property, the likely mechanism of nephroprotective activity [9]. A report suggest that quercetin has a marked protective effect on cadmium induced nephrotoxicity that results from an increase in Metallothionein, a small cysteine-rich protein and eNOS (endothelial nitric oxide synthetase) expression and the inhibition of Cox-2 (Cyclooxygenase-Z) and iNOS (inducible nitric oxide synthetase) expression [16]. The effect of aqueous extract of Beekh Kasni was better than methanolic extracts. Therefore further investigation using specific fractions of the extracts can help to isolate and identify potential nephroprotective agents.

Conclusion

Present study was designed to investigate nephroprotective effect in Gentamicin induced nephrotoxicity in albino rats. A significant reduction of serum creatinine and serum urea even at a very high dose of gentamicin showed that the test drug possesses a significantly high level of nephroprotective effect. The findings of the present study corroborate the description of Unani literature as Beekh Kasni has been described to possess nephroprotective effect [2-4]. In our study following were the main observations:

- Beekh Kasni possesses striking nephroprotective effect in Gentamicin induced nephrotoxicity.
- Since, Gentamicin produce toxicity at different sites of nephron, therefore it was inferred that BK can be effective against different toxins that can produce injury/toxicity at different parts of kidney.
- Test drug was also found to possess curative effect as it produced significant effect in the animals pretreated with test drug. It demonstrated therapeutic potential of test drug along with its ability to induce nephroprotection.
- It is interesting to note that aqueous extract of the test drug was found to be more significant than the methanolic extract suggesting that it can be effectively used in therapy in the same dosage form as described in Unani Medicine.
- The study validated the claim of ancient Unani practitioners, about the use of Beekh Kasni in the management of renal diseases.

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