Nephroprotective activity of *Sinapis alba* in gentamicin induced murine model of renotoxicity

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**ABSTRACT**

*Sinapis alba*, belongs to the family Brassicaceae and is known to possess an antioxidant capacity. Based on this background, this study was planned to look for the nephroprotective activity of *Sinapis alba* seed extract against nephrotoxicity caused by gentamicin in rats. *Sinapis alba* seeds were dried in the shade and then powdered. Then, the powder was subjected to extraction with 95% ethanol and brought to a syrup consistency by distilling the solvent and reducing the pressure using a water bath. Animals were divided into four groups. Each group had six animals in it. Group 1 serves as normal control, while the next group was disease control (received gentamicin). The rats in third and fourth groups were treated with the extract of *Sinapis alba* (200 and 400 mg/kg). The animals were sacrificed, and biochemical and histopathological studies were carried out after the 8th day of study. Results of the study showed positive effects with 200 mg/kg as well as 400 mg/kg doses of *Sinapis alba* extract. Serum urea, creatinine, and BUN was reduced significantly in the test group compared to the group which received gentamicin (p<0.05). Moreover, this biochemical evidence was also validated with the histopathological results indicated with the absence of necrosis and casts in the tubular lumen. Ethanolic extract of *Sinapis alba* administered by the oral route in an experimental murine model using gentamicin as an instigator of damage to the kidney, effectively reduced renal damage in rats, therefore provides a scientific evidence of a renoprotective effect.

**INTRODUCTION**

A large number of drugs, including prescription drugs, cause kidney injury ([Mehta *et al.*, 2004; *Uchino et al.*, 2005; *Hoste et al.*, 2015; Moffett and Goldstein, 2011; Perazella, 2012; Markowitz and Perazella, 2005; Gokhale and M, 2000]). Different studies on acute kidney injury revealed that 14-26% of acute kidney injuries (AKIs) are drug-induced. It is also seen that 16% of the patients hospitalized with acute kidney injuries are children ([Mehta *et al.*, 2004; *Uchino et al.*, 2005; *Hoste *et al.*, 2015]).

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et al., 2015; Moffett and Goldstein, 2011). The elements that contribute to the drug-induced renal injury are the innate nephrotoxic capability of the drug, the patient factors as well as the pharmacokinetics of the drugs. Aminoglycosides, amphotericin B, cisplatin, methotrexate, tenofovir, amiloride, and NSAIDs are few of the many that cause acute renal failure.

Aminoglycosides are attributed as the commonly known cause of drug-induced kidney injury. They account for 10-15% of all AKI (Pelte et al., 1981) and studies reveal that 30% patients on gentamicin treatment for greater than 7 days develop nephrotoxicity (Kotra et al., 2000). Aminoglycosides antibiotics have a wide spectrum of action and exhibits its bactericidal activity by interfering with protein synthesis by binding to the A-site on the 16S component of the 30S ribosomal RNA of the bacteria (Eliopoulos and Eliopoulos, 1988). They are used in therapy for many indications, including protozoan infection as solo agents or in combination with other antibiotics (Avent et al., 2011; Jackson et al., 2013).

The use of aminoglycosides declined with the advent of other classes of wide-spectrum antibiotics, which showed enhanced improvement in their safety profiles, but with the appearance of multidrug-resistant organisms, efforts are being made to achieve better therapeutic indices of this class of drugs (Gilbert Dn et al., 1995; Krause et al., 2016). An effort to reduce toxicity has been achieved through risk evaluation, recognition, response, renal support, rehabilitation, and research (Awdishu and Mehta, 2017). Risk evaluation of a patient who are predisposed for drug toxicity, alteration of a treatment regime to meet patients need along with efficient monitoring have certainly reduced the side effects to a large extent (Walker and G, 1988; Moore et al., 1987; Prins et al., 1996; Smith et al., 1980), but the safety is low when compared with other classes of drugs. Therefore, the research is targeted to obtain ways to combat the causatives of toxicity.

Antioxidants are said to be protective against renal damage. It is seen that various drugs cause damage to kidneys by affecting antioxidant levels. This could be due to the cellular damage caused by them. Lowering of the antioxidant levels allows reactive oxygen species (ROS) to cause damage to the cells, which in turn leads to nephrotoxicity. Hence methods that mitigate the effects of these oxygen species may be useful in preventing acute renal failure. This can be done by consuming dietary or pharmacological antioxidants, which will reduce oxidative stress (Singh et al., 2006).

In recent years, many plants have been investigated to explore the probable source of antioxidants as they provide significant health benefits (Zengin et al., 2011). Phenolics, mainly flavonoids and phenolic acids in plants, possess the antioxidant property to scavenge free radicals of oxygen as they have the capacity to donate an electron (Velioglu et al., 1998). Sinapis alba, commonly referred to as white mustard, belongs to the family Brassicaceae is one such plant which is known to have the antioxidant capacity (Ishida et al., 2014).

Sinapis alba are cultivated in India and are available commercially as mustard seeds and have become the primary ingredient in many recipes. They are known to possess antibacterial, antifungal, antiproliferative effect and acts as an appetizer, stimulant, emetic, as well as relieves flatulence, increases sweating, promotes diuresis, improves digestive, promotes sputum secretion (Aboulfadl et al., 2011). Phytochemical investigations on Sinapis alba revealed the presence of phenolic groups such as p-hydroxybenzoic acid, sinapic acid, sinapine, and glucosinolate such as sinalbin (Al and Shalini, 2016). Sinapine is the dominant phenolic compound and may possess the highest antioxidant property.

Since no data is available on the renoprotective activity against acute renal injury by Sinapis alba, the current study was to planned to seek for the protective activity of extract of Sinapis alba seeds against kidney damage in rats by gentamicin treatment.

MATERIALS AND METHODS

Plant Material
Sinapis alba seeds was purchased from the local market of Udupi. Authentication was provided by Dr. Gopalkrishna Bhat, a retired botanist at Poorna Prajna College, Udupi. Seeds were collected in the month of September, and the dried seeds were stored in airtight containers until use.

Drugs and chemicals
Gentamicin sulfate (Piramal Health Care Ltd) was purchased from the hospital pharmacy, and all the other chemicals were purchased from Sri Durga Laboratory Equipment Supplies, Mangaluru.

Method of preparation of ethanolic extract
Sinapis alba seeds were dried in the shade. The dried seeds were then powdered with an electronic mixer. The powder was filled into the Soxhlet apparatus in small quantities of 200 grams per load and was further used for extraction with 95% ethanol for 30-40 hours. After extraction, it was brought to a syrup consistency by distilling the solvent from...
the extract, and reducing the pressure using a water bath (<50°C). It was dried using a desiccator.

**Animals**

For this experiment, adult female Wistar albino rats weighing around 200g were used. Animals were accommodated under standard environmental settings and were provided with standard diet and water. The study protocol was approval by the Institutional Animal Ethics Committee prior to start of the project.

**Acute Toxicity Study**

The acute toxicity study of *Sinapis alba* extract was carried in adult female Wistar albino rats using the limit dose of 2000mg/kg under the Organization for Economic Co-operation and Development (OECD) guidelines 425 (*Acute Oral Toxicity, 2008*). After dosing, each animal was observed for general behavior and mortality once during the first 30 minutes, then regularly during the first 24 hours and then every day subsequently for 14 days in total. There was no death. From this study, a dose of up to 2000mg/kg was considered safe, and based on the results, 200mg/kg and 400mg/kg doses were used for the study.

**Experimental protocol**

**Gentamicin induced nephrotoxicity in rats**

After acclimatization, four groups of 6 animals each were made in random and placed in separate cages. The animals were categorized into 4 groups,

Group 1 - Normal saline was administered, and this group was used as a control for the study.

Group 2 - Received gentamicin (80 mg/kg) as intraperitoneal injections during the study and used as disease control and (*Kumar et al., 2000*).

Group 3 - Received gentamicin (80mg/kg/day) intraperitoneal injections and the test drug - *Sinapis alba* at a dose of 200mg/kg/day.

Group 4 - Received gentamicin (80mg/kg/day) intraperitoneal injections and the test drug - *Sinapis alba* at a dose of 400mg/kg/day per orally.

The test drug *Sinapis alba* was started three days preceding the intraperitoneal injections of gentamicin and was continued with the gentamicin treatment for eight days.

**Sample collection and biochemical assays**

**Plasma markers of renal damage**

The rats were anesthetized with ketamine + xylazine, 24 hours after the last dosing. Blood was collected by cardiac puncture, centrifuged at the rate of 1500 cpm for 15 minutes to isolate serum. The animals were then sacrificed. The right kidneys were isolated and treated for further histopathological examination. Urea and creatinine was measured from serum. Other parameters estimated were serum uric acid and blood urea nitrogen.

**Histopathological examination**

10% neutral buffered formalin was used to fix the isolated kidney. After further processing, it was embedded in paraffin. With the help of a microtome, 5mm sections were made. Hematoxylin and eosin was employed to stain the sections. Evaluation of the sections was done under a light microscope and was scored as follows (*Kumar et al., 2000*)

- Score 0 for the normal renal section with no signs of necrosis or cell casts.
- Sections showing focal areas (<1% of total tubule population) with granulovacuolar epithelial cell degeneration and granular debris in lumens of the tubule with or without tubular epithelial cell desquamation are scored as 1.
- Sections that involve less than fifty percent of proximal tubules with epithelial necrosis and desquamation are given a score of 2. When sections show more than fifty percent of proximal tubules with necrosis, it is scored 3. Score 4 is when there is complete (or almost) necrosis of the tubules.

**Statistical analysis**

The data obtained was expressed as mean ± SEM (standard error of the mean). The statistical test to analyze the differences between different groups was One-way ANOVA, followed by Tukey’s test. The value of *p* < 0.05 was taken as statistically significant in this study.

**RESULTS AND DISCUSSION**

**Effect of *Sinapis alba* extract body weight**

There was a noticeable increase in the mean body weight of the rats fed with *Sinapis alba* extract. While normal control and the disease control group didn’t show any significant changes.

**Effect on biochemical variables**

Group 2 animals, which received gentamicin (80mg/kg) for eight days duration, showed substantial (*p* < 0.05) increase in serum urea (57.83±3.13mg/dl), creatinine (1.92±0.17/dl), and BUN (26.3±1.03mg/dl) as compared to Group 1, which were normal control animals (Table 1).

Group 3 showed a significant decrease in serum urea (20.16±6.48), creatinine (0.8±0.25), BUN (6.8±2.96) in comparison to Group 2.
Figure 1: Histopathology of kidney sections of normal control showing normal renal tubular morphology. (H & E staining; 10X magnification)

Figure 2: Histopathology of kidney sections of gentamicin treated group showing dilated tubules with flat tubular epithelium with pyknotic nuclei and granular casts in the lumen and presence of widespread inflammatory cells in the interstitium (shown by arrow). (H & E staining; 40X magnification)

Figure 3: Histopathology of kidney sections showing the effect of Sinapis alba (200mg/kg and 400mg/kg) in the gentamicin treated group. Less than half the proximal tubules involved showing mild tubular dilatation. No signs of necrosis or casts in lumen. The interstitial damage is also absent. (H & E staining; 40X magnification)

Group 4 showed a significant decrease in serum urea (20.83±6.48), creatinine (0.75±0.25), BUN (9.06±1.76) when compared to Group 2.

Group 3 and 4 didn't reveal any significant changes in uric acid in comparison to group 2.

Therefore, we can infer that Group 3 and Group 4, that received Sinapis alba, showed renoprotective effects at doses 200mg/kg and 400mg/kg body weight.

Histopathological evaluation

The kidney sections of the rats were studied under the microscope and scored as follows

Group 1 rats treated with normal saline revealed normal tubular morphology with intact glomerulus with a score of 0. (Figure 1)

Group 2 rats treated with gentamicin showed acute kidney injury characterized by dilated tubules, denuded epithelium, extensive tubular necrosis with granular casts, glomerular and interstitial infiltration with a score of 4. (Figure 2)

Group 3 rats treated with Sinapis alba (200mg/kg/day) showed 33.3% with a score of 3 where greater than half the proximal tubules were involved and 66.67% with a score of 2 involving fewer than half the proximal tubules. The average score of the group was 2.3. (Figure 3)
Table 1: Effect of *Sinapis alba* extract on various biochemical parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Blood Urea Nitrogen (BUN) (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Nitrogen (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>37.83±4.60</td>
<td>1.05±0.13</td>
<td>17.63±2.16</td>
<td>2.12±0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin (80mg/kg)</td>
<td>57.83±3.13*</td>
<td>1.92±0.17*</td>
<td>26.3±1.03*</td>
<td>2.04±0.35*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin (80mg/kg) + <em>Sinapis alba</em> (200mg/kg)</td>
<td>20.16±6.48**</td>
<td>0.8±0.25**</td>
<td>6.8±2.96**</td>
<td>0.26±0.43**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin (80mg/kg) + <em>Sinapis alba</em> (400mg/kg)</td>
<td>20.83±6.48**</td>
<td>0.75±0.25**</td>
<td>9.06±1.76**</td>
<td>0.69±0.43**</td>
<td></td>
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</tr>
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</table>

Values are expressed as mean ± standard error of mean; *p < 0.05 in comparison with normal control group and **p<0.05 in comparison to a group receiving gentamicin.

Group 4 rats treated with *Sinapis alba* (400mg/kg/day) showed 83.3% with a score of 2, involving less than half the proximal tubules (Figure 3).

The results obtained from biochemical as well as the histomorphological section, providing evidence of nephroprotection afforded by *Sinapis alba* as they could reduce the dreadful effects of gentamicin.

The present study was an attempt to look for the beneficial outcome of *Sinapis alba* in renal damage induced by gentamicin in a murine model.

The present study noted an appreciable weight gain in groups fed with *Sinapis alba*. This finding is substantiated by previous studies, which reveal *Sinapis alba* as an appetizer and a stimulant (Aboulfadl et al., 2011).

Intraperitoneal administration of gentamicin at 80mg/kg/day dose resulted in significant renal damage as an evidenced increase in serum urea (57.83±3.13mg/dl), creatinine (1.92±0.17/dl), and BUN (26.3±1.03mg/dl) as compared to Group 1, which were normal control animals. Group 2 showed extensive tubular necrosis along with with dilated tubules, granular casts, as well as interstitial infiltration in the kidney sections, which agreed with earlier studies that describe gentamicin, induced nephrotoxicity (Kumar et al., 2000).

The hallmark of aminoglycoside-induced nephrotoxicity is tubular necrosis; it produces oxidative stress in cells of the tubules mediated by hydroxyl radicals from H$_2$O$_2$ and superoxide anion. Several experiments have suggested cell damage in the kidney due to gentamicin is by enhancing reactive oxygen species (ROS) production. This is accompanied with depletion of antioxidant enzymes in the renal tissues (Maldonado et al., 2003; Walker et al., 1999; Zorov, 2010). ROS damages proteins and also alters the cell membrane integrity through lipid peroxidation. This leads to numerous structural and functional changes (Sahu et al., 2013). Therefore, antioxidants have been a major focus of research interest as a means to reduce or prevent the renal damage caused by gentamicin.

The other features include disruption of the basal membrane, contraction of mesangial cells, cell proliferation, and apoptosis with marked alteration of intra-glomerular hemodynamics. Earlier studies have shown that the morphological changes induced by aminoglycosides involves cellular edema, interruption of the basal membrane, thinning of the Bowman’s capsule, and features suggestive of acute necrosis of the tubules.

In the present study, *Sinapis alba*, when administered at doses of 200mg/kg and 400mg/kg body weight, has shown considerable nephroprotection by decreasing the serum urea, creatinine, and BUN levels significantly when compared with the group which received only gentamicin. It has prevented a rise in serum urea, blood urea nitrogen, and creatinine levels due to gentamicin. Moreover, this biochemical evidence is also validated with the histopathological results, which is indicated with the absence of necrosis and casts in the tubular lumen.

Medicinal plants and its products have been a major source of natural antioxidants, which offer a more effective and less toxic alternative to synthetic antioxidants. The presence of the antioxidant activity of *Sinapis alba* is well documented in previous studies.
CONCLUSIONS

Taking into consideration the above result revealing the functional and histological renal changes, we conclude that the ethanolic extract of *Sinapis alba* administered by the oral route in an experimental murine model using gentamicin as an instigator of damage to the kidney effectively reduced renal damage in rats, therefore, provides a scientific evidence of a renoprotective effect. However, further detailed research is essential to investigate the mechanisms by which *Sinapis alba* exerts its renoprotective effects and its usefulness in humans exposed to gentamicin.

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Authors’ contributions

All six authors were actively involved either in the designing and execution of the research work or both. All the authors have extended their help in the final drafting of the manuscript.

Conflict of interest

Nil.

Ethical considerations

The study was executed after receiving approval from the institutional animal ethics committee clearance. (KMC/MNG/IAEC/15-2018). The experiment was carried out, giving utmost importance to all ethical and animal handling guidelines.

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