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NEPHROPROTECTIVE ACTIVITY OF *TRACHYSPERMUM AMMI* SEEDS IN MICE

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ABSTRACT

Ajowan *(Trachyspermum ammi)* is a popular kitchen spice which is routinely used as home remedy for the management of a wide range of GIT disorders. From ancient times, Ajowan is employed as a nephro-protective agent, but there is no sufficient scientific work has been done in this area. So, present study is designed to explore the nephro-protective potential of *Trachyspermum ammi* seed powder (TASP). A total of 60 swiss mice were employed in this study. Kidney damage was induced by administering gentamicin (800 mg/kg, *i.p.*) once daily for 10 days. TASP was incorporated along with diet for successive 10 days simultaneously at 0.5% w/w, 1.0% w/w and 2.0% w/w concentrations. Levels of serum cretanine and blood urea nitrogen (BUN) were estimated. The mechanism of action was studied by estimating malondialdehyde (MDA) and glutathione (GSH) levels in kidney homogenates in order to evaluate the degree of lipid peroxidation. Histopathological studies were also done to confirm the biochemical changes. Simultaneous treatment of TASP leaded to reversal of nephro-toxicity induced by gentamicin as indicated by significant fall in blood urea nitrogen (BUN) and serum creatinine. Histopathological studies revealed that the TASP reversed the structural degeneration induced by gentamicin administration. TASP has also reversed the lipid peroxidation indicated by significant decrease in MDA level and increase in GSH level. From the study, it can be sum up that the TASP protected the kidneys from gentamicin damage. Probable mechanism of action is by protection against oxidative damage by gentamicin.

Keywords: *Trachyspermum ammi*, Ajowan, Kidney, Nephro-protective, Gentamicin.

INTRODUCTION

Trachyspermum ammi Linn commonly known as Ajowan is an important member of Indian kitchen. Ajowan seeds are used to impart its distinct taste and flavor to a number of culinary dishes. Traditionally, the Ajowan seeds have been used in India as a folk remedy for arthritis, asthma, coughs, diarrohea, indigestion, intestinal gas, influenza and rheumatism [1]. *T. ammi* is reported to possess hypotensive [2], antiplatelet [3], antihyperlipedimic [4], anti-inflammatory [5], analgesic [6], hepatoprotective [2], bronchodilatory [7], spasmolytic [2], antitumor [8], antibacterial [9], antifungal [10] and antiviral [11] activities. *T ammi* seeds are rich in terpenoids, minerals, vitamins and fatty acids [12]. Protein derived from Ajowan seeds are reported to have anti-calcifying property and therefore reduced the renal stone formation in rats [13].

Gentamicin is an antibiotic having nephrotoxicity as its major side effect. Gentamicin is rapidly excreted by glomerular filtration and mainly reabsorbed in the proximal tubules leads in accumulation within the renal cortex; this binding is responsible for gentamicin-induced nephrotoxicity [14]. The mechanism by which gentamicin induces nephrotoxicity involve oxidative and nitrosative stress. Gentamicin induces superoxide anions and hydroxyl radical production from renal mitochondria [15]. In addition, peroxide generation [16], lipo-peroxidation [17], and that of reduced glutathione is diminished [18] in renal cortex from gentamicin-treated animals. Further, the

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scavenging of reactive oxygen metabolites provide protection against nephrotoxicity induced by gentamicin [19]. Hence, the present study was designed to explore the effect of *Trachyspermum ammi* seeds on gentamicin-induced nephrotoxicity in mice.

MATERIALS AND METHODS

Plant Material

Dried seeds of *Trachyspermum ammi* were collected from the local market of Hisar (Haryana), India. Seeds were pulverized using mechanical grinder. The powdered form was stored in an air tight container. This *Trachyspermum ammi* seeds powder (TASP) was administered orally for 10 successive days in three different doses (0.5 %, 1.0 % and 2% w/w) by admixing with the standard diet. The doses of TASP were determined on the basis of a pilot study and literature reports. The animals were given the food *ad libitum*. The diet intake was measured daily by weighing the remaining diet (uneaten) in the cages and subtracting this amount from the total feed amount given on the previous day.

Animals

All the experiments were carried out using male, Swiss mice procured from disease free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. Young (3-4 months old) mice weighing around 20g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12h each) light-dark cycle. Food given to mice consisted of wheat in the form of dahlia boiled in water with small amount of salt and refined oil. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. The experimental protocol was approved by the Institutional Animals Ethics Committee and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number - 436).

Drugs:

Gentamicin sulphate (Garamicin 80 mg/2 ml ampoule, Schering-Plough, U.S.A.), Dose 80 mg/kg body weight, intraperitoneally, daily for 10 days.

Experimental Design:

A total of 60 mice were divided into 5 equal groups (n=12).
Group 1 (Control): No drug treatment, only simple feed.
Group 2 (Gentamicin alone): Gentamicin for 10 days.
Group 3 (TASP 0.5% + Gentamicin): TASP 0.5% w/w incorporated with diet and gentamicin injection for 10 days.
Group 4 (TASP 1.0% + Gentamicin): TASP 1.0% w/w incorporated with diet and gentamicin injection for 10 days.
Group 5 (TASP 2.0% + Gentamicin): TASP 2.0% w/w incorporated with diet and gentamicin injection for 10 days.

Twenty-four hr after last drug administration, the animals in each group were divided into two subgroups.

Assay of Serum Urea and Creatinine

The animals of first subgroups were sacrificed for collection of blood and centrifuged at 3000 rpm for 10 min . to measure serum urea and creatinine using diagnostic kits of Erba laboratories.

Histopathological Observation

After the blood was collected, sections were taken from each kidney immediately. The tissue was fixed in 10% neutral formalin for a period of at least 24 hr, dehydrated in graded (50~100%) alcohol and embedded in paraffin, cut into 4~5 µm thick sections and stained with haematoxylin-eosin for photomicroscopic assessment [20].

Estimation of Lipid Peroxidation in Kidney Tissue

Lipid peroxides as malondialdehyde (MDA) were measured spectrophotometrically (UV-VIS Systronics Spectrophotometer) after the reaction with thiobarbituric Acid Reactive Substances (TBARS). Briefly, the animals of second subgroups were sacrificed for separation of kidneys. After ice-water washing of the tissue, the kidney was weighed and homogenized in 9 volumes buffered saline (0.9% w/v) in a tissue homogenizer and analysed for MDA generation as nmol MDA/g tissue [21].

Measurement of Glutathione in the Kidney Tissue

Reduced glutathione (GSH) was assayed by spectophotometric technique using Ellman's reagent [22]. This method is based on the reductive cleavage of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) by sulfhydryl (SH) group to yield a yellow color with maximum absorbance at 412 nm. Briefly, the tissue was washed with phosphate buffer pH 7.0. A part was weighed and homogenized with 5% trichloroacetic acid (TCA), ethylene diaminetetraacetic acid (EDTA). It was then centrifuged at 3000×rpm for 15 min. at 4 °c in the cooling centrifuge. The supernatant was separated and used for GSH analysis. The level of reduced GSH was calculated as µmol GSH/g wet tissue.

Statistical Analysis

The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. Values are expressed as mean ± SEM. P values <0.05 were considered significant.

RESULTS

Effect of TASP Administration on Biochemical Parameters of Mice

Gentamicin (80 mg/kg, *i.p.*) induced prominent kidney damage as indicated by a significant increase in serum urea and creatinine when compared to control group (Figure 1 & 2). Simultaneous treatment with TASP with gentamicin for 10 days, significantly protected the kidneys

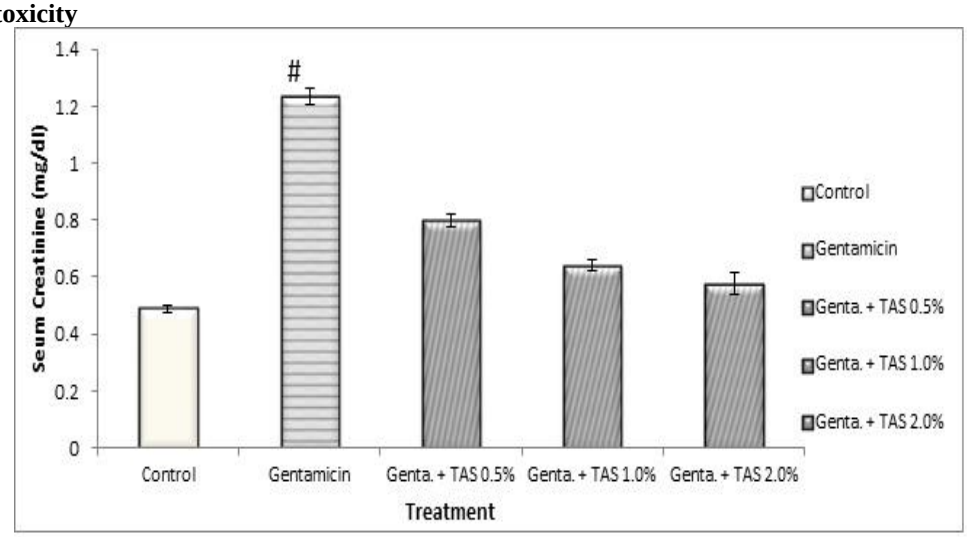
tissue from damage, as deduced from the significant decrease in serum urea and creatinine (Figure 1 & 2).

Moreover, administration of gentamicin resulted in a significant increase in the level of MDA and a significant decrease in GSH content in the kidney homogenates (Figure 3). Simultaneous feeding of TASP with gentamicin administration, significantly reversed the gentamicin induced decrease in the level of GSH and increase in the MDA content in the kidneys homogenates (Table 1).

Effect of TASP Administration on Histopathology of Mice

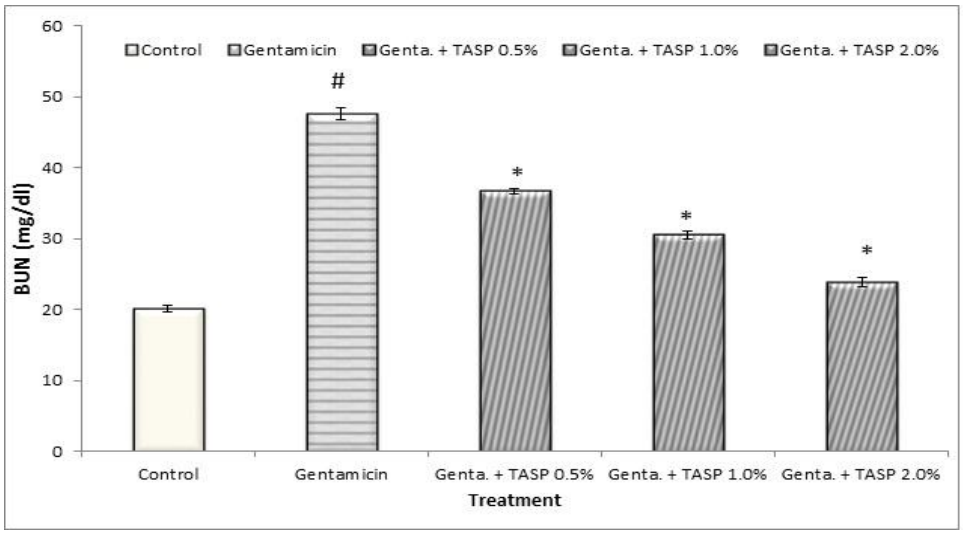
Histological sections from gentamicin group showed acute tubular necrosis and glomerular widening. TASP at concentration of 0.5% w/w showed focal necrosis of the proximal convoluted tubular lining pithelial cells with areas of desquamation of the cells in the tubular lumina whereas TASP at concentration of 1.0 % w/w and 2.0 % w/w showed reversal of gentamicin induced necrosis of the proximal tubular lining epithelial cells along with cellular swelling, desquamation and loss of brush border (figure 3).

Figure 1. Effect of TASP (0.5%, 1.0% and 2.0%) administration on Serum Creatinine level (mg/dl) in Gentamicin induced nephrotoxicity



Values are represented as Mean ± SEM (n = 12); Gentamicin (80 mg/kg *i.p.*) was used as negative control drug; One way ANOVA followed by Dunnett's t-test; ; * indicates $p < 0.01$ v/s Gentamicin group; # indicates $p < 0.01$ v/s control; TASP = *Trachyspermum ammi* seeds powder; Genta. = Gentamicin

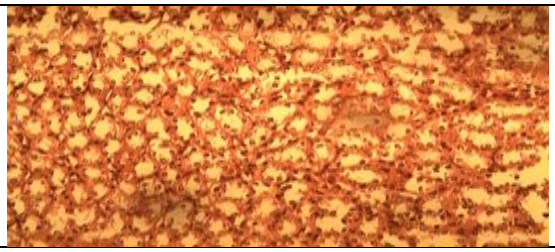
Figure 2. Effect of TASP (0.5%, 1.0% and 2.0%) administration on BUN (mg/dl) in Gentamicin induced nephrotoxicity



Values are represented as Mean ± SEM (n = 12); Gentamicin (80 mg/kg *i.p.*) was used as negative control drug; One way ANOVA followed by Dunnett's t-test; * indicates $p < 0.01$ v/s Gentamicin group; # indicates $p < 0.01$ v/s control; TASP = *Trachyspermum ammi* seeds powder; BUN = Blood Urea Nitrogen; Genta. = Gentamicin.

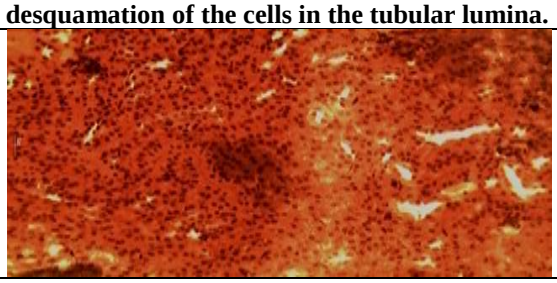
Figure 3. Effect of TASP (0.5%, 1.0% and 2.0%) administration on tissue histopathology of kidneys of mice having Gentamicin induced nephrotoxicity

Control group showed normal glomerular and tubular histology. The tubules were largely intact without the presence of any mononuclear infiltrates in the interstitium and blood vessels were also unremarkable

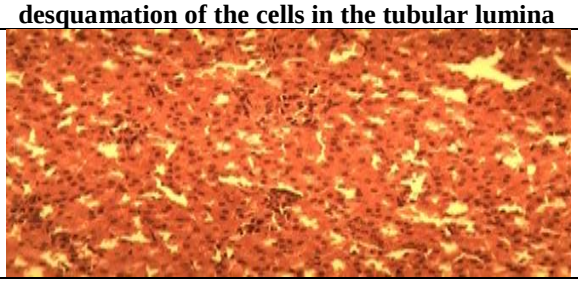


Negative control (gentamicin) group showed acute tubular necrosis and glomerular widening.

TASP at the concentration of 0.5 % w/w showed small recovery of focal necrosis of the proximal convoluted tubular lining epithelial cells with narrowing of areas of desquamation of the cells in the tubular lumina.



TASP at the concentration of 1.0 % w/w showed better recovery of focal necrosis of the proximal convoluted tubular lining epithelial cells with narrowing of areas of desquamation of the cells in the tubular lumina



TASP at the concentration of 2.0 % w/w resulted in almost recovery of focal areas of necrosis of the proximal tubular lining epithelial cells as well as lessen cellular swelling, desquamation and loss of brush border

Table 1. Effect of TASP (0.5%, 1.0% and 2.0% w/w) administration on MDA and GSH level in Gentamicin induced nephrotoxicity

Group	MDA (nmol/g of tissue protein)	GSH (µmol/g of tissue protein)
Control	0.48 ± 0.017	4.29 ± 0.05
Gentamicin	1.73 ± 0.059#	2.72 ± 0.066#
Genta. + TASP 0.5%	1.678 ± 0.053*	3.45 ± 0.065*
Genta. + TASP 1.0%	1.245 ± 0.09*	3.77 ± 0.044*
Genta. + TASP 2.0%	1.11 ± 0.033*	3.89 ± 0.046*

One way ANOVA followed by Dunnett's t-test; * indicates $p < 0.01$ v/s Gentamicin group; # indicates $p < 0.01$ v/s control; TASP = *Trachyspermum ammi* seeds powder; MDA= Malondialdehyde; GSH = Reduced Glutathione; Genta. = Gentamicin.

DISCUSSION

Gentamicin is a well-established model for nephrotoxicity used extensively in scientific studies [23-27]. Recent evidence suggests a role of free radicals induced oxidative stress in gentamicin toxicity [28-30]. Gentamicin was found to induce an abnormal rise in generation of superoxide anions and hydroxyl radicals production from renal cortical mitochondria. Hence, oxygen free radicals so generated play an important role in pathogenesis of nephrotoxicity by gentamicin. In present study, we have observed that gentamicin induced significant increase in kidney content of MDA, while the level of GSH was significantly decreased. Furthermore, gentamicin significantly increased serum urea and

creatinine level. A number of studies reported that antioxidants are effective against gentamicin-induced nephrotoxicity [29, 31-34].

A number of earlier studies, reported the significant antioxidant profile of *Trachyspermum ammi* seeds [35-37]. Feeding of TASP simultaneously with gentamicin protected the kidney tissue as indicated from the significant decrease in serum urea and creatinine and MDA content in the kidneys homogenates (Figure 1, 2 and Table 1). Moreover, the level of GSH in the kidney homogenates was significantly enhanced (Table 1). These observations show that addition of ajowan seeds in the daily diet of mice has protected the gentamicin induced nephrotoxicity.

The protective effect of TASP against gentamicin was also studied using histopathology of the kidneys of the mice (figure 3). The TASP at the concentration of 1.0 % and 2.0 % w/w was found to be effective in the reversal of gentamicin induced tissue necrosis and swelling of cells. These observations from histopathology studies confirm the biochemical changes induced by TASP.

Previous study reported that Ajowan seed powder feeding with diet (2%, 4% and 6% w/w) leads to decrease in MDA and increase in reduced glutathione level [38] in DMBA-induced skin and B(a)P-induced forestomach papillomagenesis. Similarly this study also revealed that feeding of Ajowan seeds powder along diet protects kidney from oxidative damage as indicated rise in GSH level and fall in MDA level (Table 1). A recent study revealed that protective activity of *T. ammi* seeds aqueous extract against gentamicin-induced nephrotoxicity in albino rabbits. On the same line, in our study also, feeding of Ajowan seeds powder as a dietary component lead to protection against gentamicin induced nephrotoxicity probably due to its strong antioxidant profile.

CONCLUSION

In conclusion, in our study, Ajowan seeds is found to possess good potential to act as nephroprotective agent against gentamincin induced toxicity which might be due to strong antioxidant profile of it. Hence, receiving Ajowan seeds as a daily dietary component may prove helpful in the management of kidney disorders. Further clinical studies should be carried out to support these observations of pre-clinical studies.

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