

## Bioavailability enhancement studies of amoxicillin with *Nigella*

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**Background & objectives:** *Nigella sativa* Linn. is extensively used in the Indian diasporas as spice, which may interact with co-administered drugs and affect their intestinal availability. The purpose of this study was to investigate the effect of *Nigella* on bioavailability of amoxicillin in animal model.

**Methods:** Everted rat intestinal sacs were used for *in vitro* experiment to study the transfer of amoxicillin across the gut. Amoxicillin (6 mg/ml) was co-infused with 3 and 6 mg of methanol and hexane extract of *Nigella* seeds separately. The amount of amoxicillin that traversed the gut was followed spectrophotometrically at 273 nm. For *in vivo* studies Wistar albino rats were used. Amoxicillin (25 mg/kg, po) was co-administered with hexane extract of *Nigella* seeds (25 mg/kg, po). The amount of amoxicillin in rat plasma was determined by UPLC-MS/MS method.

**Results:** The *in vitro* studies both with methanol and hexane extracts of *Nigella* increased the permeation of amoxicillin significantly ( $P < 0.001$ ) as compared to control. Permeation was also found to be significantly higher for the hexane extract ( $P < 0.001$ ) in comparison to methanol extract at the same dose levels. *In vivo* experiments revealed that  $C_{max}$  of amoxicillin in rat plasma when administered orally alone and in combination with hexane extract increased correspondingly from  $4138.251 \pm 156.93$  to  $5995.045 \pm 196.28$  ng/ml while as  $AUC_{0-t}$  increased from  $8890.40 \pm 143.33$  to  $13483.46 \pm 152.45$  ng/ml.h.

**Interpretation & conclusions:** *Nigella* enhanced amoxicillin availability in both *in vivo* and *in vitro* studies. As the increase in bioavailability is attributed, in part, to enhanced diffusivity across intestine, our study indicated that *Nigella* increased intestinal absorption of amoxicillin.

**Key words** Absorption enhancement - amoxicillin - bioavailability - everted rat sac - *Nigella sativa*

*Nigella*, belonging to *Ranunculaceae*, is a small genus of annual herb found in Southern Europe and Western Asia, especially in the mediterranean region. Three species are recorded in India. *N. sativa* Linn. is one of these species, which is found as a weed in Punjab, Himachal Pradesh, Bihar and Assam<sup>1</sup>. Its seeds are angular, dark gray in colour and are locally known

as 'Kalajira' or 'Kalongi'. Its oil is generally regarded as safe (GRAS) by Food and Drug Administration<sup>2</sup>. The seeds are considered carminative, stimulant, diuretic, emmenagogue, galactagogue, whereas its oil is applied externally for skin eruptions. It is extensively used in the Indian diasporas as spice, which may interact with co-administered drugs and affect their availability. The

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purpose of this study was to investigate the effect of *Nigella* on bioavailability of amoxicillin in rats.

Amoxicillin is a beta-lactam antibiotic classified as a class III drug with low permeability according to Biopharmaceutics Classification System<sup>3</sup>. The drug is conventionally administered as capsule or as oral suspension. The drug shows absorption through two mechanisms- energy dependent active mechanism and electro-chemical gradient dependent passive mechanism but has poor oral absorption due to complete ionization under gastrointestinal pH conditions and exhibits very low lipid solubility<sup>4</sup>. In rat plasma profile, the drug has revealed low bioavailability<sup>5</sup>. It was also confirmed by the reports obtained from a regional perfusion technique *in vivo* in humans<sup>6</sup>. Among the reasons for poor oral bioavailability of drugs, the permeability of intestinal epithelium plays an important role in passive transport. Several factors seem to influence the permeability of intestine and thereby affect the absorption of substances by the gut. These include herbs, chemicals, stress, inflammation, toxins and probiotics<sup>7</sup>.

In the present study the effect of concomitantly infused *Nigella* extracts on the absorption of amoxicillin was studied using *in vitro* models. As a model for the *in vitro* absorption enhancement studies excised rat intestinal segments were used. Amoxicillin permeation with hexane extract was significantly higher than methanol extract in *in vitro* studies and was thus evaluated in *in vivo* studies.

### Material & Methods

**Plant materials:** *Nigella* seeds were collected from Green Earth Products Pvt. Ltd, Delhi, India. Samples were identified by Dr H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), Delhi. A voucher specimen with reference number NISCAIR/RHMD/1147/179/01 has been retained in the herbarium for future reference.

**Chemicals:** Amoxicillin was obtained as gift from Ranbaxy Pvt. Ltd., Gurgaon, India. Potassium dihydrogen orthophosphate, sodium hydroxide, acetonitrile (LC-MS grade, Assigned purity 99.9%; Lot No: 90525) and ammonium acetate were purchased from Sigma-Aldrich, Germany. Dichloromethane was purchased from Merck Specialties Pvt. Ltd. (E. Merck, India). Water used in the entire analysis was prepared in-house with Milli-Q water purification system procured from Millipore (Millipore Corporation, USA). Other chemicals used were of analytical grade from commercial sources.

**Preparation of extracts:** Seeds were cleaned, milled and then passed through a 35 mm (42 mesh) sieve. The grounded seeds (25 g) were separately extracted with hexane and methanol for 6 h using Soxhlet apparatus. The solvent was evaporated under reduced pressure. The extracts were stored at 4°C in the dark till further used.

***In vitro* experimental design and surgical procedure:** The study was performed in Phytochemistry Research Laboratory, Hamdard University, New Delhi, and the protocol was duly approved by the Institutional Animal Ethics Committee (No. 639/09). Adult male Wistar albino rats weighing 120-150 g were procured from central animal facility and housed in cages under standard laboratory conditions (10 h dark/ 14 h light, temperature 20-25 °C, relative humidity 65%) for seven days. Animals were fasted for 18 h before the experiment. After decapitation, the abdomen of rats was opened by a midline incision. The entire small intestine was removed quickly by cutting across the upper end of duodenum and the lower end of ileum, and by stripping the mesentery manually<sup>8</sup>. The small intestine was washed out with normal saline solution (0.9% w/v NaCl) using a syringe equipped with blunt end. Intestinal segments (8 ± 2 cm) were everted. After being blotted with a piece of filter paper, a glass weight (1 g) was fixed and tied to the end of everted gut segment to make an empty gut sac. This was important to prevent peristaltic muscular contractions, which may otherwise alter the shape and internal volume of the sac.

***In vitro* everted intestinal sac permeation study:** The methanol and hexane extracts were tested with amoxicillin using everted rat sac model. Wilson and Wiseman method with slight modifications was followed<sup>9</sup>. The empty sac was filled with 1 ml of amoxicillin (6 mg/ml) in PBS (pH 7.4) using a blunt-end syringe. The needle was then slipped off carefully, and the loose ligature on the proximal end was tightened. The serosal compartment contained buffer in the sac. The distended sac was placed inside organ tube of organ bath containing 50 ml PBS. This gut sac bath was surrounded by a water jacket maintained at 37 ± 0.5 °C. The mucosal fluid compartment content was continuously mixed with air bubbles using an aerator. At predetermined intervals, 5 ml of sample was withdrawn from the organ tube and same volume was replaced with fresh buffer. The concentration of drug that traversed intestinal surface was monitored at 273 nm spectrophotometrically. Experiment was repeated

with amoxicillin along with 3 mg and 6 mg of methanol and hexane extracts separately. All experiments were conducted in triplicate.

A solution of amoxicillin (6 mg/ml) was prepared in PBS (pH 7.4). PBS was prepared by mixing 250 ml 0.2 M potassium dihydrogen orthophosphate and 393.4 ml of 0.1 M sodium hydroxide, both dissolved in distilled water and final volume made up to one litre<sup>10</sup>. Amoxicillin (10 mg) was dissolved in 100 ml of PBS to prepare a stock solution 100 µg/ml. Different dilutions ranging from 10 to 100 µg/ml amoxicillin were prepared by serial dilution method. Absorbance was recorded at 273 nm<sup>11</sup>. PBS was used as blank and absorbance was plotted against concentration. Permeated concentration of amoxicillin was calculated by standard plot of amoxicillin in PBS.

*In vivo experimental design:* Adult male Wistar albino rats (200-250 g) were used for the experiments. Animals were fed on standard pellet diet (Ashirwad Industries, Chandigarh) and water was provided *ad libitum*. The rats were fasted overnight before the day of the experiment. Animals were randomly distributed in two groups (n = 6), one group received amoxicillin (25 mg/kg body weight, po) while the other group received amoxicillin (25 mg/kg b.w., po) and *Nigella* hexane extract (25 mg/kg b.w., po). Blood samples were collected from tail vein in pre-heparinized glass tubes at different time intervals post-dosing (0, 0.250, 0.500, 0.750, 1, 1.5, 2, 4, 6 and 8 h). Blood samples were centrifuged (2500×g; 10 min; 20 °C) to separate plasma fractions. The collected plasma samples were preserved in deep freezer until analysis.

*In vivo pharmacokinetic investigation:* UPLC-MS/MS method for estimation of amoxicillin in rat plasma was used<sup>12</sup> with suitable modification. The chromatographic separation was achieved on a Waters Acquity UPLC™ system (Miliford, MA, USA) equipped with Waters Acquity BEH C<sub>18</sub> column (100.0 x 2.1 mm; 1.7 µm) using isocratic mobile phase acetonitrile-ammonium acetate (85:15 v/v). For isocratic elution, the flow rate of degassed mobile phase was maintained at 0.2 ml/min and 10 µl of sample solution was injected in each run. The Waters Q-TOF premier detector was operated in multiple reactions monitoring mode via positive ionization interface using the transitions *m/z* 366.1→349.1 for amoxicillin and *m/z* 350.1→192.1 for ampicillin used as internal standard. The recovery of the analytes from rat plasma was optimized using liquid-liquid extraction (LLE) technique in

dichloromethane<sup>13</sup>. The total run time was 3.0 min and the elution of amoxicillin occurred at 0.88 ± 0.05 min. Concentration-time curves were established for each analyte and used for the determination of pharmacokinetic parameters such as peak plasma concentration (*C*<sub>max</sub>), peak time (*T*<sub>max</sub>) and extent of absorption (AUC) by a non-compartmental analysis using PK Solutions Version 2.0; Summit Research Services, USA.

*Data analysis:* Three-group comparisons were analyzed by paired Student's two-tailed *t*-test. *P* value was calculated by ANOVA.

## Results & Discussion

The solvent extraction of *Nigella* seeds with hexane yielded a golden yellow colour with a strong aromatic odour while the methanol extract was dark brown in colour with a disagreeable odour. The percentage yield was 30.2 per cent with hexane and 39.32 per cent with methanol. The effect of these extracts on *in vitro* permeation of amoxicillin was investigated using everted rat sac model. The amounts of amoxicillin permeated alone and in combination with *Nigella* extracts were calculated. It was observed that the permeation of amoxicillin with 6 mg methanol extract increased significantly (*P*<0.001) as compared to control. Permeation enhancement with hexane extract showed dose-dependent increase and was significantly higher (*P*<0.001) than the methanol extract at comparable concentrations (Table I). Having its higher effect on permeation of amoxicillin, the hexane extract was selected for *in vivo* studies. There was a significant increase (*P*<0.001) in amoxicillin concentration in rat plasma in presence of hexane extract (25 mg/kg po). The maximum concentrations of amoxicillin achieved in rat plasma when administered orally, alone and in combination with hexane extract, were 4138.251 ± 156.93 to 5995.045 ± 196.28 ng/ml (Table II) while AUC<sub>0→t</sub> increased from 8890.40 ± 143.33 to 13483.46 ± 152.45 ng/ml.h, respectively. It indicated that *Nigella* increased the rate and extent of absorption of amoxicillin.

These results suggested that hexane extract of *Nigella* affected intestinal absorption that might be attributed to the presence of fatty acids in it. Long chain fatty acids (oleic, linoleic acid) and medium chain fatty acids (caprylic, capric acid) have been reported to increase oral bioavailability of peptides, antibiotics and other important therapeutic agents<sup>14</sup>. The oral bioavailability of cinnarizine was greatly enhanced

**Table I.** Cumulative concentration of amoxicillin permeated in presence of *Nigella* extracts in *in vitro* studies

Time (min)	Permeated concentration of amoxicillin ( $\mu\text{g/ml}$ )				
	1	2	3	4	5
15	7.39 $\pm$ 0.077	9.56 $\pm$ 0.343	14.35 $\pm$ 1.160*	15.85 $\pm$ 1.036*†	20.62 $\pm$ 1.953*†‡
30	9.05 $\pm$ 0.409	12.65 $\pm$ 1.330	21.21 $\pm$ 1.493*	17.34 $\pm$ 1.513*†	22.55 $\pm$ 1.330*†‡
45	10.52 $\pm$ 0.500	14.39 $\pm$ 1.190	28.61 $\pm$ 1.168*	23.12 $\pm$ 1.579*†	29.54 $\pm$ 1.466*†‡
60	13.91 $\pm$ 0.883	20.22 $\pm$ 1.571	35.75 $\pm$ 1.167*	28.49 $\pm$ 1.717*†	39.51 $\pm$ 1.255*†‡
75	15.13 $\pm$ 0.981	21.43 $\pm$ 1.030	44.28 $\pm$ 1.232*	33.64 $\pm$ 1.264*†	48.15 $\pm$ 1.338*†‡
90	17.53 $\pm$ 1.532	23.65 $\pm$ 1.317	51.88 $\pm$ 1.430*	38.47 $\pm$ 1.544*†	58.32 $\pm$ 1.438*†‡

1, Amoxicillin (6 mg/ml, control); 2, Amoxicillin (6 mg/ml) + methanol extract (3 mg); 3, Amoxicillin (6 mg/ml) + methanol extract (6 mg); 4, Amoxicillin (6 mg/ml) + hexane extract (3 mg); 5, Amoxicillin (6 mg/ml) + hexane extract (6 mg). The values represent mean of three observations  $\pm$  SD; \* $P$ <0.001 compared to control; † $P$ <0.001 compared to methanol extract at same doses; ‡ $P$ <0.05 compared to lower concentration of hexane extract

by oleic acid<sup>15</sup>. A concentration dependent increase in the oral bioavailability of polar high molecular weight drugs like glycyrrhizin in rats has been found with fatty acids<sup>16</sup>. Fatty acids have also been reported to produce a dose dependent increase in  $C_{\text{max}}$  of norfloxacin in rabbits<sup>17</sup>. Fatty acids act as absorption enhancers by increasing the fluidity of the apical and basolateral membranes<sup>18,19</sup>. Earlier we have studied the effect of hexane extract of *Nigella* on permeation of carvedilol and documented its fatty acid profile. *Nigella* was

found to exhibit significant enhancement in permeation of carvedilol<sup>20</sup>. Linoleic acid, oleic acid, margaric acid, *cis*-11, 14-eicosadienoic acid and stearic acid were identified as main fatty acids<sup>21</sup>. It was thus postulated that since the hexane extract contains both long and medium chain fatty acids, a synergistic effect cannot be ruled out.

In conclusion, our results indicated that *Nigella* when co-administered with amoxicillin increased its absorption across gut wall. Thus, *Nigella* was found to be efficient absorption enhancer for amoxicillin and can be tested as absorption (bioavailability) enhancing system for various low permeable drugs.

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**Table II.** Concentration of amoxicillin in rat plasma in presence of *Nigella* hexane extract after oral co-administration

Time (h)	Concentration of amoxicillin (ng/ml)	Concentration of amoxicillin with hexane extract (ng/ml)
0.000	0.000	0.000
0.250	151.780 $\pm$ 8.96	439.043 $\pm$ 28.91*
0.500	527.912 $\pm$ 23.43	992.92 $\pm$ 62.73*
0.750	2152.613 $\pm$ 91.26	3091.239 $\pm$ 139.02*
1.000	4138.251 $\pm$ 156.93	5995.045 $\pm$ 196.28*
1.500	3140.012 $\pm$ 124.29	4049.738 $\pm$ 162.67*
2.000	2049.160 $\pm$ 84.51	2904.583 $\pm$ 119.28*
4.000	982.030 $\pm$ 53.75	1604.674 $\pm$ 63.61*
6.000	241.818 $\pm$ 10.41	558.281 $\pm$ 34.75*
8.000	51.342 $\pm$ 0.991	123.015 $\pm$ 1.102*

The values represent mean of three observations  $\pm$  SD; \* $P$ <0.001 compared to control



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