

# Review on *Nothapodytes nimmoniana* and its Nephro Protective, Hepatoprotective and Anti-Oxidant Activities

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## Abstract

*Nothapodytes nimmoniana* (Graham) Mabb is small tree, 3-8 m high belonging to family Icacinaceae is commonly known as *Amruta* and found in Maharashtra, Goa, Kerala, Karnataka, Assam and Tamil Nadu in India. The major source of a potent alkaloid, namely camptothecin (CPT) and 9-methoxy camptothecin, which is used in various types of cancer, HIV, malaria, antibacterial, anti-inflammatory, antifungal activity, immune modulator. It also contains 3-ketooctadec-cis-15-enoic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Other chemical constituents isolated from this plant are acetylcamptothecin, (+)-1-hydroxypinoresinol,  $\Omega$ -hydroxypropioquaiacone, p-hydroxybenzaldehyde, scopoletin, uracil, thymine, sitosterol, sitosteryl- $\beta$ -D-glucoside. Gentamicin which is the class of aminoglycoside antibiotics (AG) are widely used in the treatment of a variety of infections (ocular, pulmonary, and intestinal infections) produced by Gram-negative bacteria and bacterial endocarditis. Their cationic structure, which depends on the number of amino groups and on their distribution within the molecule, seems to have an important role in their toxicity, mostly affecting renal (nephrotoxicity) and hearing (ototoxicity) tissues in which they accumulate. Moreover, this aminoglycoside has been widely used as a model to study the nephrotoxicity. And  $\text{CCl}_4$  is commonly used for free radical induced liver injury. Liver is not the only target organ of  $\text{CCl}_4$  but it also affects several organs of the body such as lungs, hearts, testes, kidneys and brain. It is generally accepted that  $\text{CCl}_4$  toxicity results from bioactivation of  $\text{CCl}_4$  into trichloromethyl free radical by cytochrome P450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver toxicity. The nephroprotective and hepatoprotective activity of *N. nimmoniana* is probably due to the presence of Flavanoids & Alkaloid (camptothecin) in *N. nimmoniana* plant. Hence, the review of the study is concluded that the *N. nimmoniana* may possess nephroprotective and hepatoprotective activity which gives many links to develop the future trials.

**KEYWORDS:** *Nothapodytes nimmoniana*, Nephroprotective activity, Hepatoprotective activity, antioxidant activity.

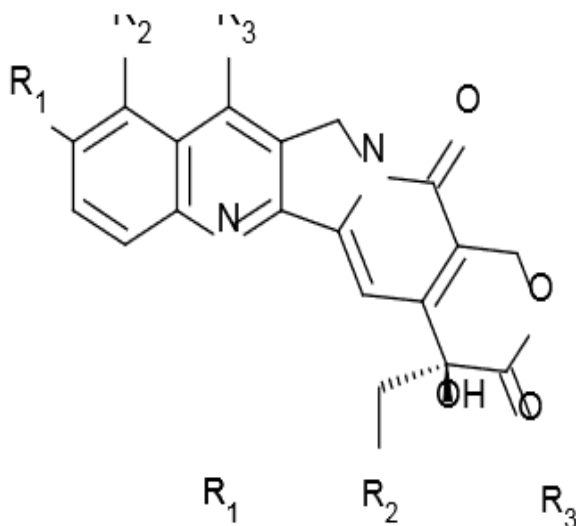
## INTRODUCTION

*Nothapodytes nimmoniana* (Graham) Mabb belonging to family Icacinaceae is commonly known as *Amruta* and found in Maharashtra, Goa, Kerala, Karnataka, Assam and Tamil Nadu in India. <sup>[1]</sup> This plant is known by different names: of *N. nimmoniana*, *durvasanemara*, *kodsa*, *hedare* (Kannada), *ghenera* (Hindi), *amruta*, *narkya*, *kalgur*, *kalagaura* (Marathi), *arali*, *choral*, *perum pulagi*, *kal kurinj* (Tamil). The major source of a potent alkaloid, namely camptothecin (CPT), which is used in various types of cancer, HIV, malaria, antibacterial, anti-inflammatory and antifungal activity have been reported. *N. nimmoniana* is one such plant which yields contain camptothecin in significantly high amount. <sup>[2]</sup> It is a small tree, 3-8 m high. Bark smooth, grey, wrinkled, about 5 mm thick. Branchlets slightly angled, corky, with prominent leaf scars. Leaves alternate, slightly leathery, broadly egg-shaped to elliptic-oblong, 1–25 x 4–12 cm, base often unequal, apex acute to acuminate, margin entire, hairless above, thinly hairy beneath, crowded at the ends of branchlets, lateral nerves 8-10 pairs, leaf stalks 3–6 cm long. Flower in cymes, creamy yellowish, foul smelling (strongly foetid), about 5 mm across, in terminal corymbose cymes, petals hairy inside. Drupes ovoid, 1.25–1.9 cm long smooth, purplish black when ripe. Seed 1 or 2, albuminous. The fruit resembles jamun or jambul fruit in taste and appearance. <sup>[3]</sup> Since there is no convenient synthetic source for CPT, we depend on raw material from natural populations. *Camptotheca acuminata* (tree of Chinese origin) and *N. nimmoniana* are the only convenient sources for large scale extraction and purification of CPT <sup>[4]</sup>. As CPT accumulates in stem and root of *N. nimmoniana*, whole tree is cut to generate biomass for extraction. In Indian market, the current demand for its biomass is 500–700 metric tons a year. In Maharashtra, overexploitation and habitat destruction for raw material has led to population decline by 50–80% in last decade. Total loss has been recorded from certain areas. Currently, the species population density is as low as 1-2 individuals/hectare in some areas. However, it extends up to 30–40 individuals/hectare at some localities such as forest of Mahabaleshwar, Satara where populations of *N. nimmoniana* survive against the severe threat of destruction <sup>[5]</sup>.



Figure 1.

Different parts of *N. nimmoniana* tree A) mature leaves with fruits, B) stems, C) immature leaves, D) mature stem



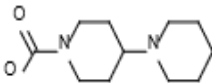
Camptothecin	H	H	H
9-Aminocamptothecin	H	NH <sub>2</sub>	H
Topotecan	OH	CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	H
Irinotecan		H	CH <sub>2</sub> CH <sub>3</sub>
SN38	OH	H	CH <sub>2</sub> CH <sub>3</sub>

Figure 2: Major chemical constituents of *N. nimmoniana*.

## N. NIMMONIANA TRADE AND IMPORTANCE IN TRADITIONAL MEDICINE

In India, research on clinical trials of CPT is conducted only at laboratory scale. Countries like Japan, USA and Spain are into the commercialization of CPT as a drug. These countries import dried raw material from India which is now one of the leading exporters worldwide. According to State Forest Department records, the annual demand from Japan for dried stem of *N. nimmoniana* was 200–300 tons in 1994<sup>[5]</sup>. Since then, there has been an increasing trend. The trade volume increased to 1600 tons in 2002. In 2006–2008, the reported trade in the volumes has exceeded 1000 tons, whereas unreported trade is thought to be at least twice the reported one. The ever-increasing worldwide market of Irinotecan and Topotecan (semi synthetic CPT analogues) has currently reached 1000 million US dollars, which represents approximately 1 ton of CPT raw material<sup>[6]</sup>. To meet this ever increasing demand for CPT related drugs from all over the world, more and more plants are being cut, dried and exported. This export business is completely managed by private sector. The collectors have trained local tribal and rural laborers in cutting and drying processes. They are paid Rs. 10-15/ kg of dried stems and exported at the price of 1500 US dollars per kg (meaning Rs. 60,000 i.e. 1000 times higher price)<sup>[7]</sup>.

Traditionally, the aqueous extract of *N. nimmoniana* has been used as anti cancer<sup>[8]</sup>. It's medicinal use has not been reported in any codified systems of Indian medicine. In fact, CPT is regarded as one of the most promising anticancer drug. In recent years, CPT has also emerged as a promising drug to be used in AIDS chemotherapy. The anti HIV activity of CPT is due to the inhibition of Tat-mediated transcription from the viral promoter. It is also active against parasitic trypanosomes and leishmania. CPT is also active against the malaria, antibacterial activity and anti-inflammatory activity from the leaves of *N. nimmoniana* Miers.

## N. NIMMONIANA: CULTIVATION AND COLLECTION

*N. nimmoniana* is cultivated in moist, deciduous place. It requires average annual rainfall of approximately 4000-7000 mm with 8–9 months of dry period. In India, it is grown under a wide range of conditions from the coastal areas up to altitude of 2300 m. In general, the crop is raised in south India's warm climate. Well drained, aerated fertile soils with a pH value ranging from 5.0 to 6.0 are the most favorable conditions for its cultivation. Seeds are used for cultivation. The seeds are shown on the seed beds in winter and early spring, the optimum temperature for germination is 12–35°C. Percentage seed germination depends on seed weight. If seed weight is high, per cent germination will be higher. *N. nimmoniana*, a tree crop, has got a 7–8-year-long gestation period. CPT yield depends on the age of plant. If the plant age is high, then per cent yield will also be higher. Highest percentage of CPT is produced by root wood, and minimum by the leaves. Collection in summer is best for obtaining CPT.

## N. NIMMONIANA: VERSATILE SOURCE OF CHEMICAL CONSTITUENTS

*N. nimmoniana* is a rich source of the potent alkaloid CPT and 9-methoxy camptothecin. It also contains 3-ketoctadec-cis-15-enoic acid (16.0%), palmitic acid (12.3%), stearic acid (4.2%), oleic acid (16.2%), linoleic acid (11.6%) and linolenic acid (39.7%). Other chemical constituents isolated from this plant are acetylcamptothecin, (+)-1-hydroxypinoresinol,  $\Omega$ -hydroxypropioquaiacone, p-hydroxybenzaldehyde, scopoletin, uracil, thymine, sitosterol, sitosteryl- $\beta$ -D-glucoside, 3- $\beta$ -hydroxystigmast-5-en-7-one, stigmast-5-en-3- $\beta$ , 7- $\alpha$ -diol, 6- $\beta$ -hydroxystigmast-4-en-3-one, sitost-4-en-3-one, linoleic acid, trigonelline, and pumiloside isolated from the stem of *N. nimmoniana* and characterized<sup>[9]</sup>. Topotecan is 4-ethyl-4,9-dihydroxy-10-[(dimethylamino)methyl]-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione; irinotecan is S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]-indolizino[1,2-b] quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, SN-38 is 7-ethyl-10-hydroxycamptothecin.<sup>[10]</sup>

## METHODS OF CPT EXTRACTION AND ISOLATION

Fulzele and Satdive reported the comparison of techniques for the extraction of the CPT from *N. nimmoniana*. Extraction methods using stirring extraction, soxhlet extraction, ultrasonic extraction and microwave-assisted extraction (MAE) were evaluated for the percentage extraction of CPT and 9-methoxycamptothecin (9-Me-CPT) from *Nothapodytes foetida*. The extracts were analyzed by HPLC. Methanol (90%, v/v) extracted high percentage extraction of CPT and 9-Me-CPT as compared to ethanol (90%, v/v). The result showed that the percentage extraction of CPT and 9-Me-CPT from *N. nimmoniana* by MAE was more efficient followed by soxhlet extraction, ultrasonic and stirring extraction methods. Maximum percentage extraction of CPT was obtained by MAE technique<sup>[10]</sup>.

Hsiao et al. determined camptothecins in DMSO extracts of *N. nimmoniana* by direct injection capillary electrophoresis. The hydrophobic compound was extracted from plant tissue with a water-miscible organic solvent, DMSO, at the elevated temperature (60°C). The extract was directly injected into the separation capillary (untreated fused silica, 34 cm in length, 75 micro m i.d.) and analyzed in MEKC mode (369 nm). Within 5 minutes of migration, camptothecins were successfully separated and quantified by adding organic modifiers to the running buffer (20% DMSO, 90 mM SDS in 10 mM borate buffer, pH 8.60). This method had been proved to be very suitable for monitoring of the amount of camptothecins during the cultivation of the medicinal plant<sup>[11]</sup>.

Yamazaki et al. reported isolation of camptothecin-related alkaloids from the methanolic extracts of *Ophiorrhiza pumila*, *Camptotheca acuminata* and *N. foetida*. Plants were profiled and identified using a reverse-phase HPLC coupled with online photodiode array detection and electrospray-ionization ion-trap mass spectrometry [12].

Puri et al. reported the separation of 9-methoxycamptothecin and CPT from *N. nimmoniana* by semi preparative HPLC. The purity of the isolates was determined by physicochemical data and liquid chromatography-mass spectrometry [13].

In the US Patent 6893668, an improved, and economical process for the isolation of CPT from the twigs and stem of *N. nimmoniana* was described which comprised of drying, grinding and hot defatting of *N. nimmoniana* twigs and stems with light petroleum fraction followed by successive sequential hot extraction with two solvents selected from CH<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, EtOAc, ether, acetone, MeOH, EtOH and CH<sub>3</sub>CN; removal of solvents under vacuum at a temperature in the range of 35-40°C, precipitation and filtration of crude extracts gave CPT with up to 0.15% yield [14].

## ***N. NIMMONIANA*: PHARMACOLOGICAL STUDIES**

### **Antimicrobial activity**

Petroleum ether, chloroform and methanol extracts of *N. nimmoniana* from leaves and stems were tested for their antibacterial activity. The methanol fractions were found to be most effective against the entire tested organism [15].

### **Antimalarial activity**

In *Plasmodium falciparum*, camptothecin trapped protein-DNA complexes, inhibited nucleic acid biosynthesis and was cytotoxic. These results provided the proof for the concept that topoisomerase I was a vulnerable target for new antimalarial drug development [16].

### **Anti-inflammatory activity**

The activities of the extracts were compared with control and standard ibuprofen. All the drugs were administered orally. When compared with petroleum ether extract, the anti-inflammatory activity of ethanolic extract was found to be more effective and 200 mg/kg dose of ethanolic extract significantly ( $p$  less than 0.01) reduced the inflammation, which was comparable with that of the standard, ibuprofen [17].

### **Immunomodulatory activity**

Puri et al. reported immunomodulatory activity of an extract of the novel fungal endophyte *Entrophospora infrequens* isolated from *N. nimmoniana*. The study evaluated the bioactivities of chloroform and methanolic extracts of *Entrophospora infrequens* with respect to their immunomodulatory potential in vitro and in vivo (in Balb/c mice). The endophyte *E. infrequens* was found to synthesize CPT, which was positively tested in chloroform. This showed for the first time the immunomodulatory potential of this neoteric CPT-producing endophyte from *N. nimmoniana* [18].

### **Antitumor/cytotoxic activity**

Luo et al. reported potent antitumor activity of 10-methoxy-9-nitrocamptothecin. The high cytotoxic potency of 10-methoxy-9-nitrocamptothecin was paralleled with its ability to increase the cellular accumulation of DNA damage. These results suggested that cell cycle regulation might contribute to the anticancer properties of 10-methoxy-9-nitrocamptothecin and strongly supported further anticancer development of 10-methoxy-9-nitro-camptothecin [19]. Huang et al. reported that CPT activated S or G<sub>2</sub>-M arrest and the homologous recombination repair pathway in tumor cells [20]. Cuong et al. demonstrated that the plant alkaloid CPT caused DNA damage by specifically targeting DNA topoisomerase, effectively devastating a broad spectrum of tumors [21].

The rate of drug-induced nephrotoxicity has been increasing with the ever increasing number of drugs and easy availability of over-the-counter medication viz. nonsteroidal anti-inflammatory drugs (NSAIDs). Angiotensin converting enzyme inhibitors, antibiotics, NSAIDs, and contrast agents are the major culprit drugs contributory to damage of kidney [22]. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various phytoconstituents. Earlier literatures have prescribed various herbs for the cure of renal disorders. Co-administration of several medicinal plants possessing the nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity [23].

Kidney is also the important target organ for the toxic effects of drugs xenobiotics and oxidative stress. Oxygen-free radicals have been implicated in several biological processes potentially important in glomerular diseases. Various environmental toxicants and clinically useful drugs, like acetaminophen and gentamicin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxides and oxygen reactive species [24].



Nephrotoxicity is the major adverse effect of different drugs. So, it is a drug induced disease. This toxicity has been induced because of the release of the oxidants in kidney. Thus, damaging or destructing the nephrons which are the basic functional units of kidney [25].

The liver is a vital organ that support nearly every other organ in the body in some facet and has a wide range of functions, including detoxification of various metabolites, protein synthesis and the production of biochemical's necessary for digestion. Because of its large metabolic conversions, it is exposed to many kinds of Xenobiotics and therapeutic agents as well as chemical compounds and environmental pollutions leading to rapidly growing morbidity and mortality from liver diseases. In spite of tremendous advances in modern medicine, no effective drugs are available which offers protection to the liver from damage or help to regenerate hepatic cells. [26] This leads to a great need for researcher to engaged searching for organ such as liver protection.

## MATERIALS AND METHODS

### Animals

Wister rats of either sex weighing 150-180gm will be used for this experiment. The animals were maintained under standard laboratory conditions in polypropylene cages under 12 hr light/dark cycle, controlled temperature(24±2°C), fed with commercial pellet diet and water in an animal house.

### Nephrotoxicity inducing agent: Gentamicin

Gentamicin which is the class of aminoglycoside antibiotics (AG) are widely used in the treatment of a variety of infections (for example, ocular, pulmonary, and intestinal infections) produced by Gram-negative bacteria and bacterial endocarditis.[27] Their cationic structure, which depends on the number of amino groups and on their distribution within the molecule, seems to have an important role in their toxicity, mostly affecting renal (nephrotoxicity and hearing (ototoxicity) tissues in which they accumulate. Moreover, this aminoglycoside has been widely used as a model to study the nephrotoxicity of this family of drugs, both in experimental animals and the human beings [28].

### Experimental Design for Nephroprotective activity.

Animals were divided into 6 groups, containing 6 animal in each.

**Group I:** Normal Control (normal saline for 10 days).

**Group II:** Diseased control (Gentamicin 80mg/kg body weight for 10 days)

**Group III:** Standard: vitamin E 250 mg/kg one hour before I.P injection of Gentamicin (for 10 days).

**Group IV:** Treated: Disease control + *Nothapodytes nimmoniana* extract (low dose) for 10 days.

**Group V:** Treated: Disease control + *Nothapodytes nimmoniana* extract (high dose) for 10 days.

Urine was collected over 24 h on 10th day by keeping the test animals in individual metabolic cages. The volume of collected urine samples was measured followed by estimation of biochemical parameters, namely urine creatinine and urine albumin. Blood samples were collected from the test animals under anesthesia (phenobarbiton sodium; 40 mg/kg of body weight; i.p) by cardiac puncture before sacrifice and serum parameters including creatinine, urea, albumin, and total protein were estimated [29,30]. The biochemical estimations were done in a Biochemical-semi-auto analyzer by standard procedures using commercial kits (Ecolin: Merck specialties, India). The kidneys were removed from the rats before sacrifice and organs were fixed using a formosal solution (10% v/v of formaldehyde in normal saline), embedded with paraffin wax followed by preparation of tissue sections using a microtome for histopathology study [31].

### Hepatotoxicity inducing agent: Carbontetrachloride

CCl<sub>4</sub> is commonly used for free radical induced liver injury. Liver is not the only target organ of CCl<sub>4</sub> but it also affects several organs of the body such as lungs, hearts, testes, kidneys and brain. It was reported from the investigation carried out on animal models of acute CCl<sub>4</sub> induced liver damage, it is now generally accepted that CCl<sub>4</sub> toxicity results from bioactivation of CCl<sub>4</sub> into trichloromethyl free radical by cytochrome P450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver toxicity.[32].

**Experimental Animals for Hepatoprotective activity:**

Animals are divided into 5 group each group consist of 6 rats.

Group-1: Normal control (normal saline for 7 days).

Group 2: Disease Control (CCL4 1.25ml/kg, i.p- 48 hr before euthanasia).

Group-3: Standard: Silymarin (for 7 days) +CCl4 (48 hr before euthanasia).

Group-4: Treated: Disease control + *Nothapodytes nimmoniana* extract (low dose) for 7 days.

Group-5: Treated: Disease control + *Nothapodytes nimmoniana* extract (high dose) for 7 days.

At the end of the treatment, rats were euthanised (as per CPCSEA guidelines), blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes. Liver was dissected out and immediately washed with ice-cold saline and a homogenate was prepared in 0.1 N Tris HCl buffer (pH 7.4) and the homogenate was used for the estimation of antioxidant marker enzymes <sup>[33]</sup>.

**Serum Biochemical Estimations:**

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/litre (IU/L). The levels of total protein, total bilirubin, cholesterol and triglyceride were estimated in the serum using standard commercial kits from (SPAN India Ltd, Surat, India <sup>[34]</sup>). Changes in body weight also recorded. Three rats per group were sacrificed, kidneys and liver were isolated from each rat <sup>[35]</sup>. The kidneys and liver were weighed and processed for Histopathological examination <sup>[36]</sup>.

**Histopathological Examination**

Both isolated organs will be cut into pieces and were kept in 10% neutral formalin solution<sup>[37]</sup> Both organs were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hemato xylin and eosin and were observed under a computerized light microscope.

**ANTIOXIDANT STUDY****Estimation of superoxide dismutase:**

Add 2.78 mL sodium carbonate buffer (0.05 mM, pH 10.2), 100  $\mu$ L of EDTA (1 mM, 0.0037 g in 10 mL distilled water). Add 20 $\mu$ L supernatant/sucrose for blank and Incubate at 30°C for 45 min. Thereafter, the reaction will initiate by adding 100 $\mu$ L of adrenaline. The change in the absorbance will recorded at 480nm for 3 min.

**Catalase estimation:**

Pipette out 100 $\mu$ L of supernatant to 1.9 mL phosphate buffer (P<sup>H</sup> 7), Add 1 mL H<sub>2</sub>O<sub>2</sub> and measured the changes at the 240 nm for three min.

**Lipid peroxidation**

1 mL homogenate will combined with 2 mL (TBA- TCA-HCl). Solution is heated for 15 min in a boiling water bath, kept it for cooling at room temp, centrifuge at 4000 rpm for 10 min. Take supernatant and measure at 532 nm.

**Estimation of Glutathione Peroxidase**

To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested with the addition of 0.5 ml of 10 % TCA, centrifuged and the supernatant were assayed for glutathione by Ellman's method.

To 2.0 ml of the supernatant, 3.0 ml disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The colour developed were read at 412 nm. Standards in the range of 200-1000  $\mu$ g were taken and treated in the similar manner. The activity was expressed in term of  $\mu$ g of glutathione consumed/min/mg protein <sup>[38]</sup>.

## CONCLUSION

It is clear that the *N. nimmoniana* play a prominent role against various diseases. The nephroprotective and hepatoprotective activity is probably due to the presence of Flavanoids & Alkaloid (camptothecin) in *N. nimmoniana* plant. Hence, the review of the study is concluded that the *N. nimmoniana* may possess nephroprotective and hepatoprotective activity which gives many links to develop the future trials. In India ayurvedic referred system, several herbs are prescribed for reducing renal and liver damage and to avoid kidney and liver related complications. These can be immense value in combating renal and liver damage. In this paper, we have attempted to use our best endeavors of *N. nimmoniana* to alternative medicine of renal and liver damage.

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