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**R E S E A R C H A R T I C L E**

# Investigation for Nephroprotective Properties of *Santalum Album* Ethanolic Extracts in Rats

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**A B S T R A C T**

On the basis of our findings, it may be to suggest that *Santalum album* has Anti oxidant activity against gentamicin induced oxidative stress in rats by decreasing the oxidative stress biomarkers Serum creatinine, serum urea in kidneys. *Santalum album* has Anti oxidant Effect, elevated by measuring Anti oxidant enzymes. There is increase in SOD in liver and kidney tissues in gentamicin induced oxidative stress in rats.*Santalum album* Has high scavenging activity against DPPH Free radicles generating system. *Santalum album* Has Nephroprotective effect against gentamicin induced toxicity in kidneys by observing the histopathological changes in rat kidney tissues. *Santalum album* Has many pharmacological activities like anticancer, antimicrobial, anti-inflammatory, analgesic, ant arthritic, antibacterial, anti-HIV, and anthelmintic activities.

**Keywords:** *Santalum album*, Nephroprotective, Anti oxidant Effect, SOD, Free radicles

**A R T I C L E I N F O**

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**CONTENTS**



## 1. Introduction

Acute renal failure (ARF) is a major complication of kidney, encountered globally. Aminoglycoside induced nephrotoxicity is one of the leading cause of ARF, accounting about 10-15% of total cases of ARF across the world. Gentamicin (aminoglycoside antibiotic) was introduced in 1963 and despite of its fatal side effects like, nephrotoxicity and ototoxicity, it has been used successfully for last 4 decades typically against gram-negative infections

because of its good bactericidal efficacy and low cost. The exact mechanism of gentamicin induced nephrotoxicity is yet to be elucidated completely. However, the etiology behind gentamicin induced nephrotoxicity is rested on the fact that aminoglycosides (gentamicin) are strong cationic drugs accumulated at biological membranes (especially at S1-S2 segments of proximal tubule) causes net increase in oxidative stress and lipid peroxidation leading to necrotic changes in renal tubules and consequently precipitates acute nephrotoxicity.

Santalum album is one of the most famous and widely used plants in perfumery and cosmetics. Apart from perfumery and cosmetics uses, sandalwood also has a wide range ofpharmacological activities and the plant can be considered as one of the important medicinalplants. Since last two decades, this plant has been studied extensively but still there is lot ofscope to exploit full potential of uses of sandalwood for mankind. Researchers across the globe have been focussing on the study of interesting chemical constituents especially sesquiterpenoids of sandalwood for more than a century with regard to their structure,synthesis and pharmacological effectiveness. With recent upsurge in research endeavors to verify the traditional healthcare uses of essential oil and their constituents by modern experimental approaches that have provided momentum to in depth pharmacological and mechanistic investigations. Various studies have established the versatile pharmacological effectiveness of sandalwood and its oil ranging from antibacterial to anticancer. It also shows prominent activity in various skin diseases. There are few toxicological studies on sandalwood. It is necessary to summaries all activities reported about this plant.

santalum album, or Indian sandalwood, is a small tropical tree, and is the most commonly known source of sandalwood. It is native to India, Indonesia, and the Malay Archipelago. Certain cultures place great significance on its fragrant and medicinal qualities. It is also considered sacred in some religions and is used in different religious traditions. The high value of the species has caused its past exploitation, to the point where the wild population is vulnerable to extinction. Indian sandalwood still commands high prices for its essential oil, but due to lack of sizable trees it is no longer used for fine woodworking as before. The plant is widely cultivated and long lived, although harvest is viable after 40 years. Etymologically it is derived from Sanskrit chandanam.

## 2. Materials and Methods

### Materials

Trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazinemethosulfate (PMS), ferrozine, glutathione reduced, bathophenanthrolinesulfonate disodium salt, Thiobarbituric acid (TBA), and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, ammonium iron (II) sulfatehexahydrate [$(NH_4)_2Fe(SO_4)_26H_2O$], 1-chloro-2,4-dinitrobenzene (CDNB), chloramine-T, hydroxylamine hydrochloride, Dimethyl-4-aminobenzaldehyde, and 2,4-dinitro phenylhydrazine (DNPH) were obtained from Merck, Mumbai, India. Ferritin was purchased from MP Biomedicals, USA. Streptomycin sulphate was obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. The standard oral iron-chelatingdrug, desirox, was obtained from Cipla Ltd., Kolkata,India.

### Methods

**Collection and Authentification of Plant Material**

The plant bark of *santalum album* collected from the mountains of tirupathy and authenticated by dr k madhava chetty, department of botany, Sri Venkateswara University, tirupathy.

**Extraction of Plant Material**

The plant heartwood is grinded in to a coarse powder with the help of suitable grinder

**Hot Continuous Extraction (Soxhlet)**

In this method, the finely ground crude drug is placed in a porous bag or “thimble” madeof strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

**Evaporation of Solvent**

The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

**Preliminary Phytochemical Screening**

Preliminary phytochemical screening of the *santalum album* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids. as per the standard methods.

**Material**

Chemicals used in the study were of analytical grade (Merck Specialties Pvt. Ltd., India). Lipoic acid (Polyherbal-reference drug; Himalya Drug Company, India), Genticyn injection (contains 80 mg of gentamicin sulphate per mL; Piramla Health Care (P) Ltd., India), biochemical estimation (urea and creatinine) kits (E-coline, Merck, India) and protein estimation kit (S.D Fine Chemicals, India) were purchased from local chemist and supplier.

**Reagents:** Benedict’s reagent, barfoed’s reagent, million’s regent, wager’s regent, Hager’s regent. Mayer’s regent.

**Animals**

The animals used in the present study were adult male wistar rats (10–12 weeks old with body weight 150-200 g), obtained from the animal house of the sanzyme bio labs pvt ltd, hyderabad. The animals were housed in colony cages, under standard laboratory conditions (12 h light, 12 h dark cycle), with free access to standard commercial diet and water. The Ethics Committee approved all experimental procedures used in the present study

**Acute toxicity studies**

**Objective of performing Acute Toxicity Studies**

The aim of performing acute toxicity studies is for establishing the therapeutic Index (TI) of a particular drug and to ensure the safety in vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals.

**Requirements**

**Animal:** wistar rats, 150-200gm

**Drugs/extracts:** extracts of *santalum album*

**Procedure**

- The overnight fasted rats were weighed and selected.
- The extracts were dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity and were observed for a period of two weeks.
- The toxic doses were selected based on the Guideline 423.

The wistar rats of single sex, weighing between 150 to 200 g were selected and divided in to 5 groups each consisting of 5 animals. They were maintained under standard conditions (room temperature at 22±3 ˚C, 12 hr light/dark) and allowed free access to water along with standard pelleted diet for one week before the experiment. The animals were subjected for acute toxicity study using each extract at a dose of 100 to 2000 mg/kg orally in 5 groups and observed at regular intervals of 1, 2, 4, 8, 12 and 24 hours for skin changes, morbidity, aggressiveness, increase oral secretion, sensitivity to the sound and pain as well as respiratory movements and mortality. And the lethal dose is selected as 2000mg/kg as animals showed severe side effects and the doses are decided as 100, 200mg/kg.

**Method of Induction**

Gentamicin, an aminoglycoside antibiotic with a wide spectrum of activities, is vastly used in the treatment of Gram-negative bacterial infections. But its usefulness is limited due to its serious side effects such as nephrotoxicity. It has been shown that up to 30% of people who receive a course of gentamicin treatment develop some symptoms of nephrotoxicity. Although the exact mechanism of gentamicin-induced nephrotoxicity is not well understood, numerous studies have found different pathways involved in this process, including production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), reduction in antioxidant defense, activation of inflammatory processes, contraction of mesangial cells, together with a decrease in renal blood flow, which lead to tubular necrosis, leukocyte infiltration, cellular damages, reduced glomerular filtration rate (GFR) and renal dysfunction. Also, it has been shown that gentamicin- induced nephrotoxicity increases numerous pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and intercellular adhesion molecule-1 (ICAM-1). Several studies have demonstrated that compounds with antioxidant and anti-inflammatory properties can reduce gentamicin-induced nephrotoxicity

**Procedure**-80mg/kg ip of gentamicin for 7 days continuously (one dose every morning).

**Experimental Animal Protocol**

**Anti-nephrotoxic activity**

Total 36 rats were divided randomly into six groups of six animals in each.

**Experimental design:**

The animals were assigned to five groups, each group containing sixrats:

**Group I:** Rats were orally administered with normal saline for 7 days as the normal control.

**Group II:** Rats received gentamicin (80 mg/kg; i.p.; once daily) for 7 days.

**Group III:** Rats were treated with gentamicin (80 mg/kg; i.p.; once daily) and treated with lipoic acid (10mg/kg) by oral for 7 days.

**Group IV:** Rats were treated with gentamicin (80 mg/kg; i.p.; once daily) and treated with EESA (100mg/kg) by oral for 7 days.

**GroupV:** Rats were treated with gentamicin (80 mg/kg; i.p.; once daily) and treatedwithEESA (200mg/kg)byoralfor 7 days.

**Evaluation parameters**

**Estimation of Superoxide Dismutase (SOD)**

0.88ml of riboflavin solution ($1.3 \times 10^{-5}$ M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60µl of O-dianisidine solution ($10^{-2}$ M in ethanol) and to this 100µl of clear separated SOD was added and optical density was measured at 460nm. Then the cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was remeasured against blank containing ethanol in place of enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine SOD.

**Estimation of serum Creatinine levels**

To 1 ml of working reagent, 50µl of serum or the creatinine standard were added and mixed.Read the absorbance after 30 sec ($A_0$) and 90 sec ($A_1$) using UV spectrophotometer at 520 nm and determine the ΔA for standard (S) and test (T). Calculate the serum creatinine in mg/dl in the test sample using the following equation.

Serum creatinine in mg/dl = (ΔAT/ΔAS)*2.

**Estimation of serum urea levels**

The entire reagents were brought to the room temperature before using the test. Undiluted serum sample was used in this method. Taken 3 set of test tubes and marked as Blank, Standard and Test. 0.001ml of serum sample was taken in test tube, 0.01ml of urea standard reagent in standard test tube, 0.01ml of distilled water was added to the Blank test tube. Added 1.0ml of urea reagent, acid reagent and DAM reagent to all the test tubes. These solutions were mixed well and kept in boiling water bath (100˚C) for 10 minutes

and cool in running tap water. Absorbance was read at 520 nm a reagent blank.

**Statistical analysis:**

All the values were expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance (ANOVA) followed by dunnett's test. P <0.05 will be considered as statistically significant.

## 3. Results and Discussion

**Percentage of Yield**

% yield of ethanol extract = (weight of extract)/(powder taken for extraction ) × 100

= 20 /200 ×100 = 10%.

% Yield of the *Murraya koenigii is* found to be **10.0**

**Table 1:** Results of Phytochemical Analysis Ethanolic Extract of *murraya koenigii*

| Name of the Phytochemical Constituents | Ethanol extract |
|---|---|
| Saponins | - |
| Alkaloid | + |
| Glycoside | _ |
| Reducing sugar | + |
| Tannin | + |
| Flavonoid | ++ |
| Steroid | _ |
| Anthocyanin | _ |
| Phenol | + |
| Amino acid | _ |
| Protein | ++ |

+: Indicates the presence and -: Indicates the absence of phytoconstituents

**Superoxide Dismutase:**

Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defence in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard. The values were shown in below table, and figure.

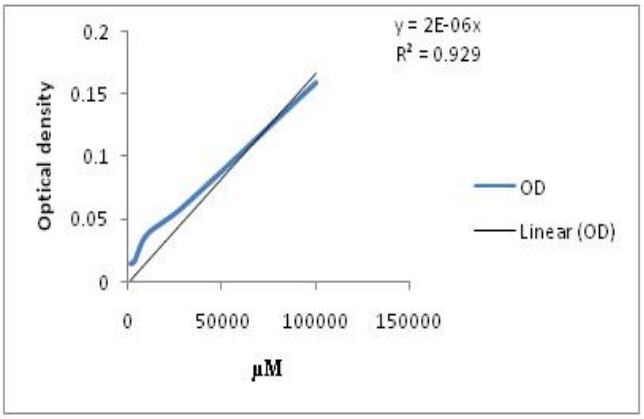


**Figure 1:** Standard graph of superoxide dismutase

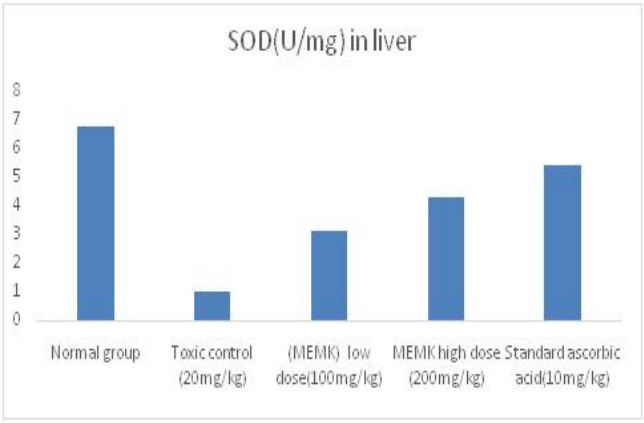


**Figure 2:** Effect of MEMK on superoxide dismutase levels in liver and kidney tissue homogenate in mice treated with AZP.

In this study, we found that 50mg/kg dose of azathioprine causes significant (p<0.001) decrease in superoxide dismutase levels. This reduction indicates that oxidative stress and toxicity is produced with azathioprine. Post treatment with MEMK at the dose of 100mg/kg and 200mg/kg after a 50mg/kg dose of azathioprine administration, shown a significant (p<0.001, p<0.0001) dose dependent increase in levels compared to toxic control group.

**Serum Alanine Aminotransferase (ALT):**

Azathioprine and test compound effects on ALT in mice from various groups shown in following figure. Measurements of ALT levels in AZP intoxicated female albino mice, and treated mice with MEMK indicate the effect of treatment. The normal control group ALT level show 132.65±1.28IU/L. After AZP treatment, the ALT level is 201.3± 22.5IU/L. This AZP treated group ALT level was increased compared to the normal control group in 21days. After 21days treatment, the *Murraya koenigii* low dose ALT level was (174.9± 9.71IU/L) decreased compared to the toxic control group has shown significance (**p<0.001) and at high dose ALT level was (150.2± 7.5IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001). On treatment standard ascorbic acid serum ALT level 141.1 ± 8.8, has shown significant (***p<0.0001).

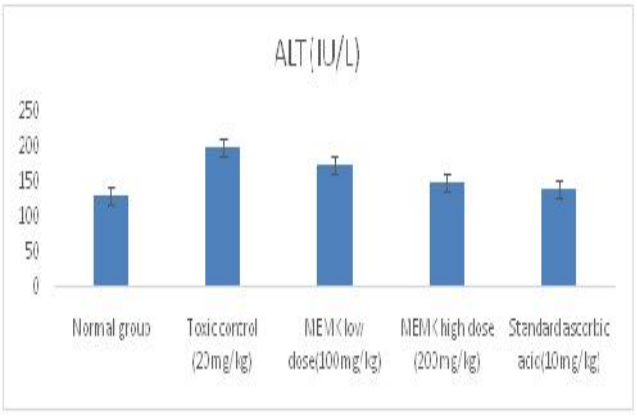


**Figure 3:** Effects of MEMK on serum ALT levels in mice treated with azathioprine

**Serum Aspartate Aminotransferase (AST):**

The above table shows the effect of test compound on serum AST levels in mice intoxicated with AZP. After 21days, the normal control group shows the AST level is 140.7±10.43IU/L. In AZP control group level is 210.5± 11.5IU/L, increased compared to the normal group. Treatment with MEMK at low dose AST level was (160.0± 10.3IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001) and at high dose AST level was (150.3± 9.92IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001). On treatment standard ascorbic acid serum AST level 141.0± 8.06, has shown significant (***p<0.0001).

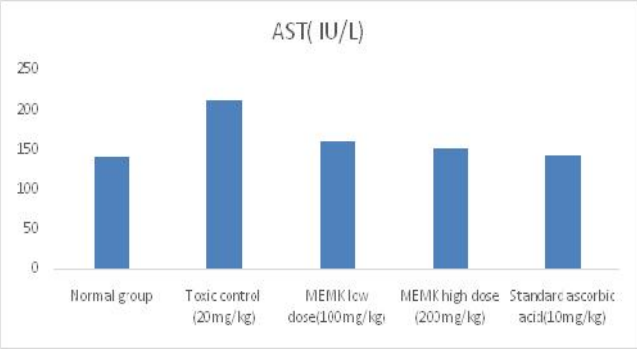


**Figure 4.: Effects of MEMK on serum AST levels in mice treated with azathioprine**

## 4. Conclusion

On the basis of our findings, it may be worthy to suggest that, *Murraya koenigii* has antioxidant activity against Azathioprine induced oxidative stress in mice by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver, *Murraya koenigii*has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in Azathioprine induced oxidative stress in mice.*Murraya koenigii*has hepatoprotective effect against Azathioprine induced toxicity in liver by observing the histopathological changes in mice liver tissue.

## 5. References

[1] Rakesh kumar nishat anjum and y.c. Tripathi, phytochemistry and pharmacology of santalum album l.: a review, world journal of pharmaceutical research, volume 4, issue 10, 1842-1876.

[2] Upul Subasinghe, Manuri Gamage, D.S. Hettiarachchi, Essential oil content and composition of Indian sandalwood (Santalum album) in Sri Lanka, Journal of Forestry Research (2013) 24(1): 127−130.

[3] Rakesh K.Sindhu, Upma, Ashok Kumar, Sahil Arora, Santalum Album Linn: A Review on Morphology, Phytochemistry and Pharmacological Aspects, Int.J. Pharmtech Res.2010,2(1).

[4] Vahid Ehsani, Morteza Amirteimoury, Zahra Taghipour, Ali Shamsizadeh, Gholamreza Bazmandegan, Amir Rahnama, Fatemeh Khajehasani & Iman Fatemi Renal Failure Vol. 39 , Iss. 1,2017

[5] Qureshi, Huma & Asif, Saira & Ahmed, Haroon & Al-Kahtani, Hassan & Hayat, Khizar. (2016). Chemical composition and medicinal significance of Santalum album: A review. Natural product research. 30. 625-639. 10.1080/14786419.2015.1036268.

[6] Hala Salah Eldin, Haidar Abdal Gadir*, Abdel Wahab Hassan, Evaluation of the hepatoprotective activity of Santalum album L, http://www.rroij.com/open-access/evaluation-of-the-hepatoprotective-activity-of-fagonia-cretica-l.php?aid=60278

[7] Matt Lam, Kirsten Wolff, Helen Griffiths, Amtul Carmichael (2014) Correction: An Aqueous Extract of Santalum album Induces DNA Damage, Cell Cycle Arrest and Apoptosis in Breast Cancer Cells via FOXO3a and p53 Expression. PLOS ONE 9(7): e102655

[8] Yarijani ZM, Najafi H, Hamid Madani S. Protective effect of crocin on gentamicin-induced nephrotoxicity in rats. Iranian Journal of Basic Medical Sciences. 2016;19(3):337-343.

[9] Nadia Abdulkarim Salih, Effect of nettle (Urtica dioica) extract on gentamicin induced nephrotoxicity in male rabbits, In Asian Pacific Journal of Tropical Biomedicine, Volume 5, Issue 9, 2015, Pages 756-760, ISSN 2221-1691, https://doi.org/10.1016/j.apjtb.2015.07.005.

[10] Mishra S, Pani SR, Sahoo S. Anti-nephrotoxic activity of some medicinal plants from tribal rich pockets of Odisha. Pharmacognosy Research. 2014;6(3):210-217.

[11] B. V. S. Lakshmi,* N. Neelima, N. Kasthuri, V. Umarani, and M. Sudhakar, Protective Effect of Bauhinia purpurea on Gentamicin-induced Nephrotoxicity in Rats, Indian J Pharm Sci. 2009 Sep-Oct; 71(5): 551–554.

[12] Naidu MU1, Shifow AA, Kumar KV, Ratnakar KS, Ginkgo biloba extract ameliorates gentamicin-induced nephrotoxicity in rats, Phytomedicine. 2000 Jun;7(3):191-7.