



## ISOLATION AND ACUTE ORAL TOXICITY STUDIES OF *ARAUCARIA HETEROPHYLLA* NOVEL NATURAL POLYSACCHARIDE GUM IN ALBINO MICE

Hema Naga Durga Divvela<sup>\*1</sup>, Lohithasu Duppala<sup>1,2</sup> and Venkata Ramana Murthy  
Kolapalli<sup>1</sup>

<sup>1</sup>A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra  
Pradesh, India.

<sup>2</sup>GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

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### \*Corresponding Author

Hema Naga Durga  
Divvela

A.U. College of  
Pharmaceutical Sciences,  
Andhra University,  
Visakhapatnam, Andhra  
Pradesh, India.

### ABSTRACT

**Objective:** The aim of present study is intended to isolate and find out the LD<sub>50</sub> for evaluate the safety of isolated *Araucaria heterophylla* natural polysaccharide gum by acute oral toxicity study in healthy albino mice of either sex as per organization for economic co-operative and development (OECD) guidelines No. 425. **Methods:** Albino mice were fasted over night, the fasted body weight of each animal was determined and dose was calculated according to the body weight. Animals weighing 160–200 g was randomly divided into 7 groups comprising of five animals each. The control group received normal saline (10mL/kg per oral) and other groups received 100, 200, 500, 1000, 1500 and 2000 mg/kg of AHG dispersion in distilled water. The animals were observed continuously for the behavioural changes for

first 30 min after dosing and observed periodically (with special attention given during the first 4 hrs) for the 24 hrs and then daily thereafter, followed for 14 days. **Conclusion:** The LD<sub>50</sub> value of isolated *Araucaria heterophylla* natural polysaccharide gum was found to be more than 2000 mg/kg body weight.

**KEYWORDS:** Acute oral toxicity, LD<sub>50</sub>, *Araucaria heterophylla* gum (AHG), and OECD guideline 425.

## INTRODUCTION

The 'science of poisons' known as Toxicology and it may be defined as the study of harmful poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. After getting an appropriate knowledge on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is recognized, this is known as its (compound) 'Bio-safety level'.<sup>[1]</sup> Diagnosis, prevention and treatment of various illnesses, is extensively practiced by the conventional and alternative medicine. In these present days, this type of medicine has been attracted public attention as it is easily accessible in some regions.<sup>[2]</sup> Treatment or prevention of diseases by natural products plays a principal role in the development of novel drug leads.<sup>[3]</sup> In primary healthcare system, phyto-therapeutic products from medicinal plants and these bioactive products from medicinal plants are recognized to be safe without any compromising health effect, and thus extensively used as self medication.<sup>[4]</sup> Therefore, additional acute oral toxicity studies are critically needed not only to recognize the range of doses that could be used subsequently, but also to expose the possible clinical signs elicited by the substances under exploration. It is also a useful parameter to investigating the therapeutic index of active drugs and xenobiotics.<sup>[5]</sup> As applicability of medicinal plants increases, experimental screening of the toxicity of these plants is important to assure the safety and efficacy of those natural sources. On the other hand, acute toxicity studies do not detect the notice effects on critical functions like the cardiovascular, central nervous, and respiratory systems which are not usually assessed during these toxicity studies and these should be evaluated earlier to human exposure.<sup>[6-7]</sup>

*Araucaria heterophylla* is a genus of coniferous tree in the family Araucariaceae. The tree grows to a height of 50-60 m, with symmetrical branches and straight vertical trunk and leaves are awl shaped, 1-15 cm long about 1 mm thick at the base on young trees and incurved 5-10 mm long and variably 2-4 mm broad on older trees. Trunk has grey brown and exfoliating in fine scales. Flowers are inconspicuous and cones are oval, 10-12 cm long and 12-14 cm diameter and take about 18 months to mature. They disintegrate at maturity to release the nut like edible seeds. In olden days the aerial parts of the tree were used in toothache and extracting teeth. The leaf powder can be used as an effective, environment friendly biosorbent for the removal of chromium (VI) and  $Pb^{+2}$  from aqueous solution. The resin extract showed antiulcerogenic activity against ethanol-induced stomach ulcers and cytotoxic activities against breast (MCF7) and colon (HCT116) cancer cell lines. Three labdane diterpenes, namely labda-18(17), 14-diene, 13-epicupressic acid and 13-O-acetyl-13-

epicupressic acid were isolated from bark exudates.<sup>[8-11]</sup> The natural gums and mucilages have been investigated for various applications as pharmaceutical excipient for design of pharmaceutical formulations.<sup>[12-16]</sup>

The present study was designed to find out LD<sub>50</sub> and to ascertain the safety of AHG by acute oral toxicity study in albino mice as per Organization for Economic Cooperation and Development (OECD) guideline 425 and the test procedure described in this OECD guideline uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity. This test procedure is of value in reducing the animal numbers required to find out the acute oral toxicity of a chemical or drug by estimation of LD<sub>50</sub> and confidence intervals, this test allows the observation of signs of toxicity.

## MATERIALS AND METHODS

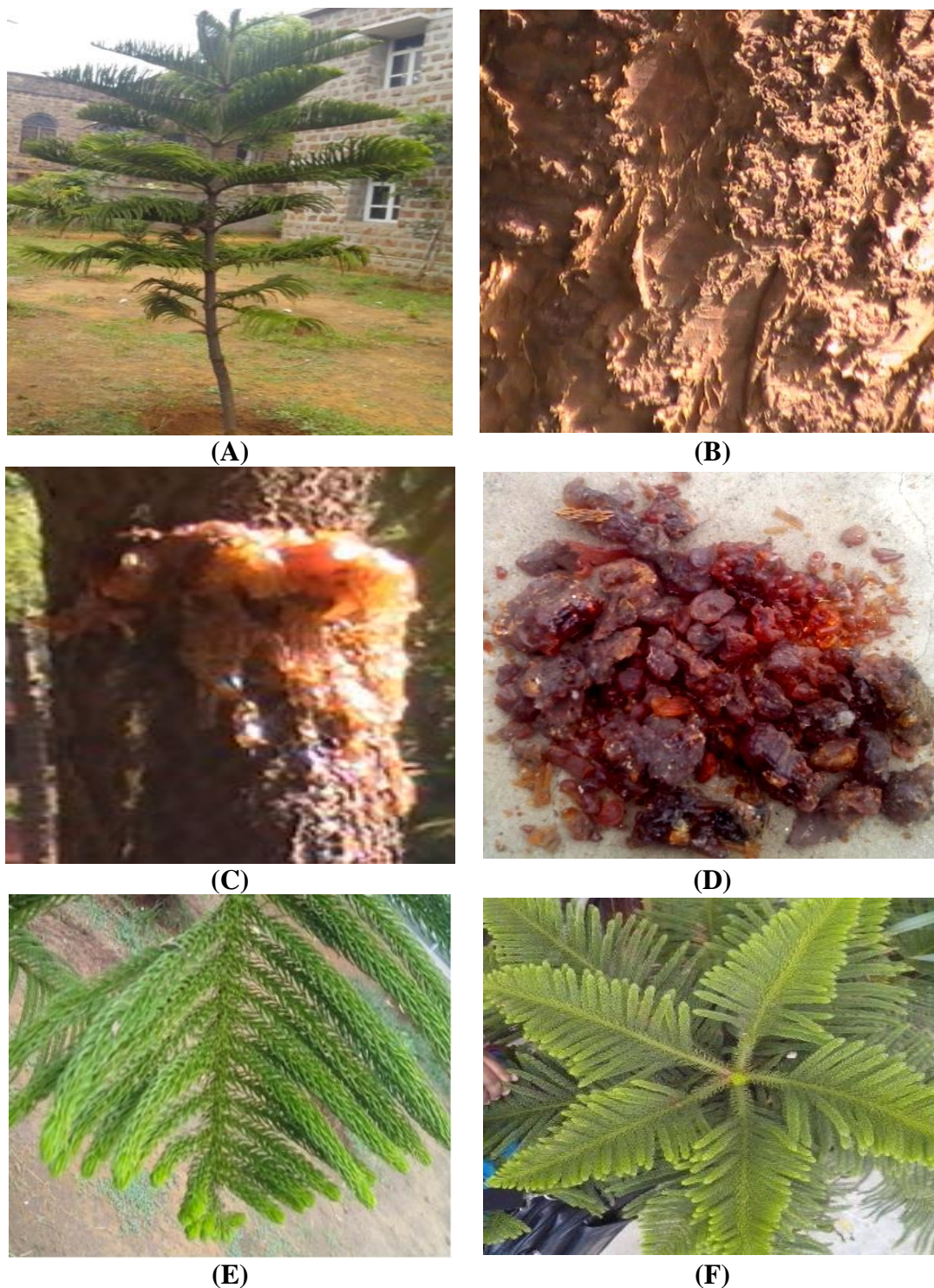
### Collection and Isolation of AHG gum

Collection of gum exudates by tapping method and initially exudates were sticky brown in colour, became harder and darker after two weeks and it was cleaned by removing the external bark and other extraneous materials by hand, finally dried in hot air oven at 50°C until it become sufficiently brittle. It was powdered by using high speed mechanical blender (Butterfly, India) and sifted through the sieve No.100 (nominal mesh aperture size 75 µm and approximate %sieving area 36). It was dispersed in distilled water in the proportion of one part of plant material to ten parts of the water. The extraction continued for 24 hrs at room temperature with continuous stirring using rotary shaker (M/s. Remi Instruments Ltd, Mumbai, India). The supernatant was separated by muslin cloth. The final residue was washed with water and washings were added to the supernatant. The procedure was repeated three times. The supernatant was collected and treated with twice the volume of acetone to isolate the gum by continuous stirring and it was separated by centrifugation process. The formed precipitate was washed with acetone and dried at 40-50°C in hot air oven. The dried *Araucaria heterophylla* gum (AHG) was pulverized by laboratory blender and passed through sieve No.100. It was stored in tightly closed container and kept in a desiccator. The percentage yield was calculated by the following equation.

$$\text{Percentage yield} = \frac{W_1}{W_2} \times 100 \quad \text{-Eq. 1}$$

Where, W<sub>1</sub> weight of crude exudates, W<sub>2</sub> weight of the isolated gum.

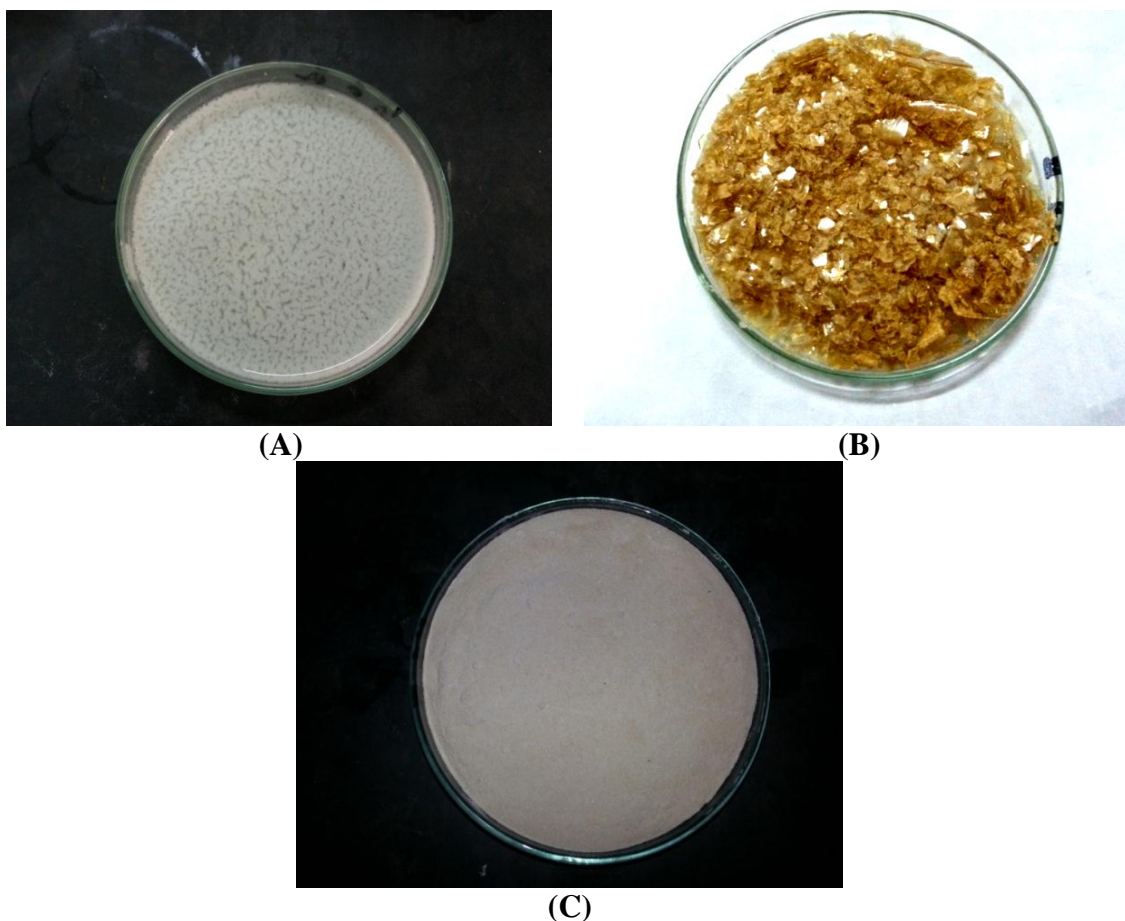




**Figure 1: Photographs of A) *Araucaria heterophylla* tree B) tree bark C) exudates D) gum tears E) foliage and F) crown.**

Gums and resins are considered as the byproducts of certain metabolic pathways. The cells lining the gum duct lumen were epithelial cells and the majority of such cells have dense cytoplasm which produces gum. When they get injured gum is secreted from the ducts. The plant was authenticated and botanically identified by Dr. S. B. Padal, Associate Professor,

Department of Botany, Andhra University, Visakhapatnam with voucher specimen No. 22203.



**Figure 2. Photographs of A) after precipitation with acetone, B) dried gum and C) gum powder after sieving**

#### **Acute Oral toxicity studies**

The acute toxicity of gum was determined as per organization for economic co-operative and development (OECD) guidelines No. 425. In acute toxicity study, determination of LD<sub>50</sub> was carried out in healthy albino mice of either sex. The present study was approved by Institutional animal ethics committee of Andhra University, Visakhapatnam (Reg. No. 516/01/A/CPCSEA). Albino mice were fasted over night, the fasted body weight of each animal was determined and dose was calculated according to the body weight. Animals weighing 160–200 g was randomly divided into 7 groups comprising of five animals each. The control group received normal saline (10 mL/kg per oral) and other groups received 100, 200, 500, 1000, 1500 and 2000 mg/kg of AHG dispersion in distilled water. The animals were observed continuously for the behavioural changes for first 30 min after dosing and observed periodically (with special attention given during the first 4 hrs) for the next 24 hrs

and then daily thereafter, followed for 14 days. All observations (changes in skin and fur, eyes and mucous membranes and behavioral pattern) were systematically recorded with individual records being maintained for each animal. Special attention was given for observation includes tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Observations and changes (if any) in wellness parameters were compared with that of control animals. The individual body weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Observations in the body weight of individual animals were noted and compared with that of the control animals.

## RESULTS AND DISCUSSION

### Collection and Isolation of plant gum

The herbarium specimen was deposited in Department of Botany, Andhra University. The exudates of plant were collected and are shown in Figure 1. The *Araucaria heterophylla* gum (AHG) was isolated by precipitation with acetone from aqueous dispersion of exudates. The percentage yield was found to be 34.38%. The precipitated *Araucaria heterophylla* gum and dried *Araucaria heterophylla* gum are shown in Figure 2. Organoleptic evaluations such as colour, odour and taste were carried by sensory organs. When *Araucaria heterophylla* gum was precipitated with acetone it was white in colour and turned to light brownish colour up on drying. *Araucaria heterophylla* gum (AHG) powder does not have characteristic taste and odour. The low concentration (1% w/v) of *Araucaria heterophylla* gum (AHG) in water formed colloidal dispersion as well as at higher concentration (20% w/v) formed thick viscous mucilage and it was insoluble in organic solvents like methanol, ethanol, acetone and ether.

### Acute Oral toxicity studies

Acute oral toxicity studies of novel natural polysaccharide gum was carried in albino mice, there was no mortality observed in all the groups indicating nontoxic nature of the AHG and the LD<sub>50</sub> value of the gum was found to be more than 2 g/kg body weight. An excipient has to fulfill the safety profile, as a drug with the exception that it should not exert a therapeutic effect. Most excipients do comply with these requirements and do have the GRAS status when used in those amounts which are normally used to fabricate drug delivery systems. As AHG did not show any acute toxicity at a dose of 2 g/kg body weight, chronic toxicity studies were not conducted.



The body weights of the animals were recorded in Table 1. There were no significant observational changes in body weight. From observation, all animals showed a normal increase in body weight without extreme difference between both control and treated groups. There is no significant changes were observed in wellness parameters (Table 2) used for assessment of acute toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated animals as well as the control animals were found to be normal and no significant changes. There is no mortality was observed.

**Table: 1 Effect of AHG on the body weight of albino mice at 2,000 mg/kg dose after 14 days.**

Group	Treatment	Body weight (g)	
		Before treatment (Mean $\pm$ SD)	After treatment (Mean $\pm$ SD)
Control	Normal Saline (10 mL/kg per oral)	163 $\pm$ 1.67	178 $\pm$ 2.78
Treated	AHG dispersion in distilled water (2000 mg/kg)	172 $\pm$ 2.89	192 $\pm$ 1.67

**Table 2: Wellness parameters observations of the treated as well as the control animals at 2,000 mg/kg body wt of AHG dispersion**

Wellness parameters	30 min		4h		1 day		2 days		7 days		14 days	
	C	AHG	C	AHG	C	AHG	C	AHG	C	AHG	C	AHG
Skin fur	N	N	N	N	N	N	N	N	N	N	N	N
Alertness	N	N	N	N	N	N	N	N	N	N	N	N
Grooming	A	A	A	A	A	A	A	A	A	A	A	A
Torch response	N	N	N	N	N	N	N	N	N	N	N	N
Pain	N	N	N	N	N	N	N	N	N	N	N	N
Tremors	A	A	A	A	A	A	A	A	A	A	A	A
Gripping strength	N	N	N	N	N	N	N	N	N	N	N	N
Pinna reflex	N	N	N	N	N	N	N	N	N	N	N	N
Corneal reflex	N	N	N	N	N	N	N	N	N	N	N	N
Pupils	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N
Skin color	N	N	N	N	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N
Hyper activity	A	A	A	A	A	A	A	A	A	A	A	A
Mortality	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	N	N	N	N	N	N	N	N	N	N	N	N

C-Control (Normal Saline), N-Normal, A-Absent, AHG-AHG dispersion in distilled water.

**Table 3: Mortality observation of AHG dispersion compared with Control for 14 days.**

<b>Group</b>	<b>Control</b>	<b>AHG dispersion</b>
60 min	NIL	NIL
120 min	NIL	NIL
180 min	NIL	NIL
240 min	NIL	NIL
Day 1	NIL	NIL
Day 2	NIL	NIL
Day 3	NIL	NIL
Day 4	NIL	NIL
Day 5	NIL	NIL
Day 6	NIL	NIL
Day 7	NIL	NIL
Day 8	NIL	NIL
Day 9	NIL	NIL
Day 10	NIL	NIL
Day 11	NIL	NIL
Day 12	NIL	NIL
Day 13	NIL	NIL
Day 14	NIL	NIL
Mortality	NIL	NIL

## CONCLUSION

The LD<sub>50</sub> value of isolated *Araucaria heterophylla* natural polysaccharide gum was found to be more than 2000 mg/kg body weight. Insignificant increase in body weight of test animals indicates that the administration of the AHG does not affect the growth of the animals. No significant changes were observed in wellness parameters as well as no mortality observed in animals. Thus, the present study confirms that AHG does not cause any apparent toxicity in an animal model.

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## Declaration of interest

The authors report no conflicts of interest.

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