

## Acute Oral Toxicity Evaluation of *Prosopis juliflora* (Mesocarp) in Sprague Dawley Rats (SDR) for Establishing Food Safety for Human Beings.

Pratibha Tewari\*, B. Dinesh Kumar\*\* and J. C. Tewari\*\*\*

**ABSTRACT:** *Prosopis juliflora* has become an important topic of discussions and policy in the country as its invasion in grasslands, protected forests and nature reserves has alarmed ecologists. Tree is playing a vital role in sustaining the livelihoods of rural poor, landless, small farmers and artisans - the least vocal groups of society. Its pods are abundantly used in coffee, confectionary, sugary mineral rich concentrate etc.; in Mexico, Argentina, Brazil, Peru, USA, etc. but prejudices and superstitions that prevail in Indian sub-continent prevents consumption of pods in any form by human beings in India. Acute toxicity analysis was therefore conducted on SDR (10M+10F), aged 6-8 weeks old, weighing 180-200gm obtained from the National Centre for Laboratory Animal Science (NCLAS) and divided in two, Group I received 2.2 gm/Kg and Group II received 4.5 gm/Kg of mesocarp suspension orally in two or three times in 24 hours. The animals were observed for mortality- activity for 14 days. Live phase of animals, cage side observation, physical, physiological and neurological parameters were monitored at regular intervals. This was followed by necropsy and histo pathological examination of all vital organs viz. brain, thymus, spleen, bone marrow, kidneys, heart, lungs, trachea, thyroid, adrenals, liver, gastrointestinal tract, testis/ovaries etc. After the experiment was over, SDR were fasted overnight and euthanized by using CO<sub>2</sub> chambers and subjected to gross necropsy of vital organs. No gross necropsy changes were observed which established the fact that *Prosopis juliflora* is safe for human consumption.

**Key words:** Food safety, acute oral toxicity, *Prosopis juliflora*, Sprague Dawley Rats (SDR)

### INTRODUCTION

*"The rebellious sands are subdued and the inhospitable soils are colonized. The dreary scene of dry districts is changed to green belts. The monotony is broken for the traveler and the sheep and goats can munch and crunch happily on the proteinous pods. The rural folk whose lands were getting buried under drifting sands are grateful to the forester and Prosopis". (Reddy, 1978).*

*Prosopis juliflora* is an ecological exotic tree of arid and semi-arid region with aggressive invasion potential to encompass large area in a short span of time. It plays a vital role in sustaining livelihoods of land less, marginal; and small farmers in arid zones. *Prosopis* species vary widely in their productivity and relative use and utilization by humans, primarily pods for food and fodder and wood for fuel and timber. The characteristics preferred by humans are the

production of large amounts of sweet pods, and rapid growth of erect trees with ability to survive and thrive in poor soils and under drought conditions (Pasezinilk *et al.* 2001). Since the mesocarp is intended for veterinary and human consumption in various ratios it becomes mandatory to undertake Pre clinical safety assessment as per Food Safety and Standard Authority Act 2006 (FSSAI) guidelines in India. *P. juliflora* has spread over in larger tracts of hot arid and semi -arid regions of the country, which constitutes 40% of it's geographical area (Tewari *et al* 2000). *P. juliflora* has become an important topic of discussions and policy in the country during recent years. Invasion of grasslands, protected forests and nature reserves has alarmed ecologists. Invasion of irrigation channels and arable land has affected the agricultural community and commercial farmers have seen their income threatened. However, *P. juliflora* is

\* Principal Scientist & Head, TOT, Training & Production Economics, CAZRI, ICAR, Jodhpur

\*\* Scientist E & Deputy Director, F&DTRC, NIN (ICAR), Hyderabad,

\*\*\* Principal Scientist & Coordinator, Cold Arid Network, CAZRI, Jodhpur

E-mail: pratibhatewari1@gmail.com

playing a vital role in sustaining the livelihoods of the rural poor, landless, small farmers and artisans - the least vocal groups of society (Roy *et al.* 2014).

Evidence suggests some Indian tribes processed pod for preparing local bread during extreme starvation conditions. Pods can be used by chewing and sucking but to process into human food, separation of pods with the mesocarp portion is essential either using hand pounding or mill. *Prosopis juliflora* with common name 'Vilayati Babool', Algaroba, Mesquite, JungleeKikar (Hindi) is one of the 44 species of *Prosopis*. It is a leguminous tree providing approximately 24 million metric tons of pods per annum worldwide (Dinesh kumar *at.al* 2014). Pods are abundantly used in coffee, confectionary, sugary mineral rich concentrate etc., in Mexico, Argentina, Brazil, Peru, USA, etc. but prejudices and superstitions that prevail in Indian sub-continent prevents consumption of pods in any form by human beings in India. As *Prosopis juliflora* pods are found abundantly in entire arid zone supporting livelihoods of people, it was felt timely to establish its preclinical safety for human consumption. It is highly justified to conduct such study in drought hit arid and semi-arid zones where finding food sometimes become difficult. In order to promote food products of mesocarp for human consumption an acute oral toxicity study in SDR was

conducted for 14 days following regulatory guidelines. The objectives of study were:

- To study the acute oral toxicity (safety) profile of *Prosopis juliflora* (mesocarp) in Sprague dawley rats.
- To promote value-addition of staple cereal foods with *Prosopis juliflora*.

## METHODOLOGY

Various line departments were visited to conduct investigations as per standard operating procedures (SOP) following principals of good laboratory practices. The institutional animal ethics committee (IAEC) of NIN, Hyderabad gave its approval for conducting the study. Sprague Dawley rats (10M+10F), aged 6-8 weeks old, weighing 180-200gm were obtained from the National Centre for Laboratory Animal Science (NCLAS) with approval of IAEC (No.P8/1-2011/BDK). All the rats were caged individually for 7 days acclimatization. This was followed by randomly dividing animals into 2 groups (Group I and Group II), Group I received 2.2 gm/Kg and Group II received 4.5 gm/Kg of mesocarp suspension orally in two or three times in 24 hours. In view of gastric emptying time the volume of suspension for administration was kept at a maximum of 2.7 ml. This exposure level was 1.1 and 2.2 times higher than the test limit of 2gm/kg. (Table 1).

**Table 1**  
**Experimental Study Design**

Species	Age	Weight	Groups	Sex		Test material concentrate #	Study period
				Male	Female		
Rats	6-8 weeks	180-200gm	Group I	5	5	2.2gm/kg	14 days
			Group II	5	5	4.5gm/kg	

Rats- The volume of administration was 1.4-2.7 ml based on body weight of animal.  
# 1.1 and 2.2 times higher than the maximum test limit of 2gm/kg respectively.

The animals were housed and placed according to group. Each animal was identified by cage label showing study title, study number, species and strain, dates of important events of the study, regular animal ID, unique ID number and group name on each cage. The mesocarp was made into a fine powder using a miller mill and a suspension was prepared by mixing 4.5gm of material in 15ml distilled water as per SOP. This was followed by administration in suspension form by oral gavage. The animals were observed for mortality- activity for 14 days. Live phase of animals, cage side observation, physical, physiological and neurological parameters were monitored at regular intervals. This was followed by necropsy and histo

pathological examination of all vital organs viz. brain, thymus, spleen, bone marrow, kidneys, heart, lungs, trachea, thyroid, adrenals, liver, gastrointestinal tract, testis/ovaries etc. Appropriate statistics was applied, data were analyzed for significant differences between the treatment groups during the experiment period.

## RESULTS AND DISCUSSION

The general behavior of all the animals in all the groups was categorized as active before and after exposure to mesocarp. Adequate water intake was found in all the groups of animals along with food. The gain in body weights was found to increase

**Table 2**  
Gain in Body Weight (Sex Pooled) of SDR

Group/observation days	Base line	3 <sup>rd</sup> Day	6 <sup>th</sup> Day	10 <sup>th</sup> Day	13 <sup>th</sup> Day
Group-I (2.2 gm/kg)	207.38± 19.366(10)	223.38± 29.033(10)	229.94± 33.499(10)	243.59± 40.707(10)	251.83± 45.596(10)
Group-II (4.5 gm/kg)	224.64± 25.027 (10)	240.73± 33.214(10)	248.30± 37.941(10)	260.48± 40.520(10)	270.05± 47.545(10)

Values are expressed as mean±SD

consistently in some animals whereas a non-significant decrease was observed in other animals. No significant treatment related effect was seen in all groups of animals during the experimentation phase.

All the animals were found sitting without any observation of purposeless circling. No abnormality in faeces excretion, consistency and color was observed. The urine color & output was normal. Animals showed no vocalization and aggressive behavior while removing from the cage.

Clean groomed hair coat, was observed in all the animals. No lacrimation, salivation, eye prominence were found. Respiration type and rate was found normal in all the animals. Eyes were found to be normal and the eyelids were open in the group of animals. Biting, convulsions and tremors were not observed, neurological disorder viz. locomotor activity, limb position, gait or pinna touch response were not observed. All the animals in the group lifted tail while walking during the observation period. Head position was normal without tilt. No change in organ weights was observed (Table 3).

**Table 3**  
Organ Weights (gm/100g) of SDR after Study Completion

Groups	Mesocarp					
	Sex pooled		Males		Females	
	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II
Parameter						
Brain	0.71±0.133(10)	0.68±0.138(10)	0.61±0.042(5)	0.56±0.081(5)	0.81±0.116(5)	0.79±0.062(5)
Heart	0.39±0.029(10)	0.39±0.038(10)	0.40±0.035(5)	0.41±0.046(5)	0.37±0.019(5)	0.38±0.025(5)
Lung	0.78±0.188(10)	0.67±0.218(10)	0.67±0.073(5)	0.62±0.123(5)	0.90±0.202(5)	0.73±0.290(5)
Liver	3.39±0.194(10)	3.35±0.557(10)	3.35±0.186(5)	3.25±0.755(5)	3.44±0.211(5)	3.46±0.317(5)
Spleen	0.27±0.065(10)	0.24±0.057(10)	0.28±0.060(5)	0.21±0.069(5)	0.27±0.076(5)	0.27±0.022(5)
Kidney(L+R)	0.71±0.200(10)	0.70±0.113(10)	0.79±0.266(5)	0.71±0.157(5)	0.63±0.060(5)	0.70±0.064(5)
Testis	1.30±0.279(10)	1.27±0.214(10)	1.30±0.279(5)	1.27±0.214(5)	0	0

After the experiment was over, the rats were fasted overnight and euthanized by using CO<sub>2</sub> chambers and subjected to gross necropsy of vital organs (Table-4). No gross necropsy changes were observed in rats which established the fact that *Prosopis juliflora* is safe for human consumption.

**Table 4**  
Necropsy Findings (Sex Pooled)

SDR Sex	Group-I		Group-II	
	M	F	M	F
No. of Animals	5	5	5	5
No. dead during treatment	0	0	0	0
No. of organs not collected due to purification	0	0	0	0
No. finally sacrificed	5	5	5	5
No. examined for gross pathology	5	5	5	5
No. showing gross pathology	0	0	0	0
Visceral organ pathology	5	5	5	5

## CONCLUSION

Pods of the species are high in sugar and protein, and are rich feed source for livestock and alternative food source for human beings. Pods are processed to prepare products like, biscuits, prepared by adding 25% of the pod powder with 75% of refined wheat flour along with sugar and baking powder to make a dough, in which essence and egg are added. This mixture is extruded into shapes and baked to obtain the products. These products can be used for family use and local market. Experiments carried out at CAZRI, Jodhpur on lactating cattle for 3 years showed that milk yield increased to the tune of 20-25% by providing *Prosopis juliflora* pods based cheaper concentrate mixture. The pods of *P. juliflora* are abundantly available in many parts of arid western Rajasthan and proper utilization of *P. juliflora* pods

for livestock concentrate ration can overcome the fodder deficit in this part of the country where man: livestock ration is 1: 1.75 and even more in many places.

The *Prosopis juliflora* mesocarp fed to Sprague dawley rats recorded no acute oral toxicity evidence as the animals remained active throughout the period of study, no pre-terminal deaths were observed. There were no abnormalities in live phase, physical activities and neurological activity throughout the study period. There was no significant difference in body weight of animal which received 2.2gm/kg and 4.5gm/kg of test material. All the animals were active throughout the study period, there were no gross necropsy changes. The test material has proved to be safe for human consumption and its processed food products can enormously impact the food market. The biscuits prepared from *Prosopis juliflora* mesocarp has potential to generate employment.

#### REFERENCES

Acute oral toxicity (safety) of *prosopis juliflora* -Mesocarp in swiss albino mice & SpargueDawley rats (2014) Vol.

I, FDTRC, NIN, ICMR & NAIP (CAZRI\_ICAR) collaborative study. Pp. 84.

Dinesh kumar, B., Tewari, Pratibha., Tewari, J.C. and Roy, M. M. (2014), Pre clinical safety evaluation of pods and mesocarp of *Prosopis juliflora* (Abstract) submitted for Asian Nutrition Congress.

JC Tewari, PJC Harris, LN, Harsh, K, Cadoret, NM, Pasiecznik (2000), *Managing Prosopis juliflora (Vilayati babul)- A Technical Manual*. CAZRI, Jodhpur, India and HDRA, Coventry, UK. 96p.

Pasiecznik. N. M., Felker, P., Harris, P. J. C., Harsh, L. N., Cruz, G., Tewari, J. C., Cadoret. K. and Maldonado, L. J. (2001), *The Prosopis juliflora- Prosopis palliada Complex: A Monograph*, HDRA, Coventry, UK.162 p.

Roy M. M., Tewari J. C., Tewari, P., Mathur B. K., Kalia R.K., Sirohi A. V. S. and Ratha K. P. (eds.) (2014), *Value Chain on Value Added Products Derived from Prosopis juliflora*, Final Report, Component II of NAIP, ICAR, CAZRI, Jodhpur, 108 p.

Reddy, C.V.K. (1978), *Prosopis juliflora, The precocious child of the plant world*. Indian Forester. 104: 14-18.