

Acute Oral Toxicity Evaluation of *Prosopis Juliflora* in Swiss Albino Mice (SAM) for Establishing Consumption Safety of Mesocarp for Human Beings.

Pratibha Tewari¹, B. Dinesh Kumar², J.C. Tewari³ & M.M. Roy⁴

*It took me long time to develop
Sustainable life support cover for human beings,
now, that I am here, I am not going to end.*
Prosopis juliflora

INTRODUCTION

Prosopis juliflora is an ecological exotic tree of arid and semiarid 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. 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, Junglee Kikar (Hindi) is one of the 44 species of *Prosopis*. It is a leguminous tree providing approximately 24 million metric tons of pods per annum world wide (Dinesh kumar et.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 swiss albino mice was conducted for 14 days following regulatory guidelines. The objectives of study were:

¹ Principal Scientist, CAZRI, ICAR, Jodhpur

² Scientist E & Deputy Director, F. & DTRC, NIN (ICAR), Hyderabad,

³ Principal Scientist & Coordinator, Cold Arid Network, CAZRI, Jodhpur

⁴ Director, CAZRI (ICAR), Consortium Leader, Jodhpur

- To study the acute oral toxicity (safety) profile of *Prosopis juliflora* (mesocarp) in swiss albino mice for livelihood support.
- To promote inclusion of 20-25% *Prosopis juliflora* mesocarp in place of staple cereals as foods for human consumptions.

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. Swiss albino mice (16M+16F) aged 4-6 weeks old, weighing 18-20gms were received from the National Centre for laboratory Animal Sciences (NCLAC). All the mice were caged individually for ten days acclimatization. This was followed by randomly dividing the animals into two groups (group I & group II). Group I received 3.2gm/kg and Group II received 6.4gm/kg of powdered mesocarp suspension orally two/four times in 24 hours. In view of gastric emptying time the volume of suspension for administration was kept at a maximum of 0.4ml. This exposure level was 1.3 and 3.2 times higher than the test limit of 2gm/kg. (Table-1)

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 histopathological 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

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. There was a constant increase in body weight of some animals. (Table-2).

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. Biting, convulsions and tremors were not observed, no neurological disorder viz. locomotor activity, limb position, gait or pinna touch response or lifting of tail was observed (Table-3). No change in organ weights was observed.

Table 1
Study Design

Species	Age	Weight	Groups	Sese		Test material concentrate#	Study period
				Male	Female		
Mice	4-6 weeks	18-20gm	Group I	8	8	3.2gm/kg	14 days
			Group II	8	8	6.4gm/kg	

Mice- The volume of administration was 0.2-0.4 ml based on body weight of animal.

1.6 and 3.2 times higher than the maximum test limit of 2gm/kg respectively.

Table 2
Mice body weight (sex pooled)

Group/observation days	Base line mean±SD	1 st Day	5 st Day	8 th Day	12 th Day
Group-I(3.2gm/kg)	23.78	23.59	24.58	25.13	24.87
	±	±	±	±	±
	2.533 (16)	2.504 (16)	2.400 (16)	2.273 (16)	1.811 (16)
Group-II(6.4gm/kg)	23.88	23.45	24.68	24.73	24.85
	±	±	±	±	±
	1.721 (16)	1.571 (16)	1.741 (16)	2.220 (16)	1.665 (16)

Table 3
Mice organ weights(g/100g)

Groups	Mesocarp					
	Sex pooled		Males		Females	
	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II
	Parameter					
Brain	1.40	1.48	1.42	1.46	1.39	1.51
	±	±	±	±	±	±
	0.224 (16)	0.267 (16)	0.239 (8)	0.303 (8)	0.222 (8)	0.241 (8)
Heart	0.60	0.62	0.63	0.68	0.58	0.54
	±	±	±	±	±	±
	0.201 (16)	0.200 (16)	0.202 (8)	0.191 (8)	0.211 (8)	0.205 (8)
Lung	0.88	0.73	0.92	0.73	0.84	0.72
	±	±	±	±	±	±
	0.255 (16)	0.173 (16)	0.198 (8)	0.155 (8)	0.312 (8)	0.205 (8)
Liver	5.65	4.60	5.51	5.27	5.79	3.84
	±	±	±	±	±	±
	2.236 (16)	0.846 (16)	0.587 (8)	0.386 (8)	3.213 (8)	0.472 (8)
Spleen	0.69	0.54	0.53	0.48	0.85	0.60
	±	±	±	±	±	±
	0.532 (16)	0.188 (16)	0.212 (8)	0.157 (8)	0.710 (8)	0.211 (8)
Kidney(L+R)	1.43	1.25	1.63	1.51	1.22	0.96
	±	±	±	±	±	±
	0.506 (16)	0.339 (16)	0.230 (8)	0.191 (8)	0.629 (8)	0.201 (8)
Testis	1.26	1.13	1.26	1.13	0	0
	±	±	±	±		
	0.359 (16)	0.322 (16)	0.359 (8)	0.322 (8)		

After the experiment was over, the mice were fasted overnight and euthanized by using CO₂ chambers and subjected to gross necropsy of vital organs (Table-4). No gross necropsy changes were observed in mice which established the fact that *Prosopis juliflora* is safe for human consumption.

Pods are chiefly consumed by animals; however our study paved way for developing its very safe novel value added products. Juli coffee was prepared after several repetitive trials and blended with crude coffee and chicory to prepare coffee substitute. It is estimated that minimum one third of Indian population likes coffee as a beverage i.e. around 400 million people. Average capacity of coffee processing is plant=500Kg per hour→4000 Kg per day→1460 t per year (assuming plant runs 365 days, 8 hrs a day). Average number of players, the market can sustain=73000/1460=50. At the present rate of *P. juliflora* raw pods, conventional coffee bean and chicory powder, the completely processed and packed instant “ Juli coffee” will cost only Rs. 45/100g. Our cost estimate are based by taking the cost of raw material to much higher rate in comparison to present real rates. This way *Prosopis juliflora* occurring in natural stands can be better managed by pod collection and processing with people’s participation

DISCUSSION

Though *P. juliflora* has spread over in larger tracts of hot arid and semi -arid regions of the country, which constituted 40% of it’s geographically 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 also playing a vital role in sustaining the livelihoods of the rural poor, including the landless, small farmers and artisans - the least vocal groups of society (Roy et. al 2014). 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 into flour to process some product like coffee substitute, highly nutritious syrup for family use and local market, and for processing some confectionery items. 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.

In a comprehensive study at CAZRI, Jodhpur on *P. juliflora* pod based products for livestock and human consumption, and their value chain in which 250 households in four villages of Jalore district of Rajasthan were involved, it was concluded that an employment of 5400 man days/year was generated through pod collection and primary value addition activities by the farmers. The processor, especially of *P. juliflora* pod based livestock feed generated employment to the tune of 1825 man days/ year and earned net profit of Rs. 1.2 million/ year. The total value of this value chain was Rs. 9.3 million/ year. These figures indicated that how much profitable can be *P. juliflora* pod based products trade if it can be extended throughout arid and semi-arid tropics of the country. *P. juliflora* trees play a vital role in the ecology and economy of many arid zone of the world where they are found. *P. juliflora* play an integral part in several sustainable land use systems that are improving livelihoods of rural population in arid and semi-arid zones while

Table 4
Necropsy findings (Sex pooled)

Sex	Group-I		Group-II	
	M	F	M	F
No. of Animals	8	8	8	8
No. dead during treatment	0	0	0	0
No. of organs not collected due to putrefaction	0	0	0	0
No. finally sacrificed	8	8	8	8
No. examined for gross pathology	8	8	8	8
NO. showing gross pathology	0	0	0	0
Visceral organ pathology	8	8	8	8

preventing further soil degradation and assisting land reclamation (Pasiiecznik et al 2001).

CONCLUSION

The *Prosopis juliflora* mesocarp fed to swiss albino mice 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 3.2gm/kg and 6.4gm/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 coffee prepared from *Prosopis juliflora* mesocarp has potential to generate employment.

References

- Acute oral toxicity (safety) of prosopis juliflora –Mesocarp in swiss albino mice & Spargue Dawley 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, Pasiiecznik (2000) *Managing Prosopis juliflora (Vilayati babul)- A Technical Manual*. CAZRI, Jodhpur, India and HDRA, Coventry, UK. 96p.
- Pasiiecznik. 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.