https://doi.org/10.48047/AFJBS.6.15.2024.5518-5527

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

ACUTE ORAL TOXICITY OF 'MANGO SEED KERNEL FLOUR' IN WISTAR ALBINO RATS

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Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.5518-5527

ABSTRACT:

Fruit and vegetable by-products are very rich in bioactive components and are found to be a potential source of antioxidants. Hard data says that they have medicinal effect on health. Mango (Mangifera indica L.) is one of the most important tropical fruits whose by-product is rich in phytochemicals. This study was carried out to evaluate the acute oral toxicity (LD_{50}), if any of mango seed kernel flourIt may be suitable for value-added products and nutraceuticals. A total of 9 wistar albino female rats were used in groups of three females for each step. The doses were orally administered with 300, 2000 & 5000 mg/kg body weight as per OECD-423 Guidelines. The treated animals were closely observed for incidence of mortality and clinical signs of intoxication periodically at one hour interval for 24 hours and twice daily for a period of 14 days. No abnormal clinical signs were recorded and none of the experimental animals died during the period of observation. Conclusively, it may be considered safe to use mango seed kernel powder, since it didn't show toxicity till 5000 mg/kg body weight.

KEY WORDS: Mango, Acute toxicity, Wistar albino rats, Antioxidants, Mango seed.

1. INTRODUCTION

In recent years, ayurveda has become an indigenous system of medicine. Due to the efficacy of ayurvedic drugs in curing several diseases with no adverse effects if properly administered, interest has developed in this area. Hence, there is need to explore effective, freely available and economical herbal drugs. Plants are immense source for discovering new products with medicinal value for usage in drug development. Traditionally, tribal people of India consumed mango seed kernel in roasted form during starvation, as it is rich in starch **(Rajan et.al, 2012).** Mango fruit is a source of plant, the king of fruits. Mango seeds are treated as waste and generate a source of pollution, but it is the most enriched part of fruit as it contains average of 6% protein, 11% fat, 77% carbohydrate, 2% ash and 2% crude fiber. Mango seeds have low protein; however have high value of leucine, valine and lysine amino acids **(Rai et.al, 2020).**

The medicinal plants are generally considered to be safe, but even they are not entirely free of side effects / toxicity. The toxicity of medicinal plants varies with the chemical composition which can arise from acute / chronic exposure. Toxic plants can affect a whole range of organ system with dysfunctions of the liver and kidneys in humans **(Anywar et.al, 2021).** Few previous studies have stated that mango seed kernel extract has been found to be protective effect against histological changes in Pb- acetate hepatotoxicity due to its higher levels of phenolics and free radical scavenging activity by **El Makawy et.al (2015). Oyedeji et.al (2018)** studied about toxicity of mango seed kernel oil which showed that oils were not toxic and have good percentage emulsion capacity; hence mango seed oil is safe for commercial and domestic utilization. **Ashalatha M (2015)** studied acute oral toxicity of mango seed powder on mice for 14 days and showed no mortality. Thus, this mango seed powder didn't show any toxic effect till 5000 mg/kg body weight. However, the identities of toxic effect of Dussheri mango are largely unknown. Hence, the purpose of this study was to examine clinical signs, mortality, necropsy finding and histopathological changes of acute oral toxicity of **"MANGO SEED KERNEL FLOUR"** in Wistar albino rats and to find out cut-off LD_{50} of test compound.

2. MATERIAL AND METHODS

2.1 Preparation of mango seed powder

Mango fruit was collected from the local market of Uttar Pradesh, Meerut in the month of June-July, 2022. The seeds were washed thoroughly under the tap water and then the seed cover was removed. After removing the hard cover, seeds were soaked for 48 hours and after soaking, they were kept in sun light to dry for 3-4 days. Dried seeds were roasted and grinded in mixer for converting it into powder. To enhance its fineness, ground powder was sieved in muslin cloth and then the prepared powder was stored in air tight container till testing.

2.2 Preparation of animals

The complete testing for the acute oral toxicity of mango seed kernel flour was done in"***Toxicology Department,Institute for Industrial Research &Toxicology"***. The female wistar albino rats were randomly selected in groups of three females each. They were approximately 200gm in weight and 8 to 12 weeks in age. The identification of rats was done by their cage tag and corresponding color body marking. The rats were fasted overnight prior to treatment and food was offered three hours after dosing. The animal room was air conditioned with temperature between 22 to 25°c and relative humidity 50 - 60%. Pelleted feed supplied by Pranav Agro Industries Ltd. (B7/6 Ramesh Nagar, Delhi India) was given in diet adlibitumand filtered water was kept in PVC bottles.

2.3 Dose level and Justification

Dose level was selected by the step wise manner: 300, 2000 & 5000 mg/kg body weight, as per ***OECD-423*** guidelines for testing of chemicals as presented in table 1& 2. Doses were prepared freshly few minutes prior to dosing and it was administered by oral route with help of oral canola at the dose volume of 10 ml/kg body weight.

Table 1: Animal Dose Administration

Group	Dose	Dose Volume	Animal No.	Gender	Day 0	Animal Dose Volume (ml)
Step 1	**300 mg/kg body weight**	**10 ml/kg body weight**	**01**	**F**	205	2.05
			02		198	1.98
			03		208	2.08
Step 2	**2000 mg/kg body weight**	**10 ml/kgbody weight**	**04**	**F**	205	2.05
			05		208	2.08
			06		206	2.06
Step 3	**5000 mg/kg body weight**	**10 ml/kg body weight**	**07**	**F**	212	2.12
			08		208	2.08
			09		212	2.12

Example: Body weight of Animal = 205g

$$\textbf{Dose Volume (ml)} = \frac{\textbf{Body Weight of animal (g) x fixed dose volume (ml/kg)}}{\textbf{1000 (g)}}$$

$$\text{Dose Volume (ml)} = \frac{205(\text{g}) \times 10\ (\text{ml/kg})}{1000\ (\text{g})} = 2.05 \text{ ml (rounded to 2.0 ml)}$$

Table 2: Dose Design

Steps	Dose (mg/kg)	Wistar albino female rats
		Nos. per group
Step I	Test compound 300 mg/kg body weight	3
Step II	Test compound 2000 mg/kg body weight	3
Step III	Test compound 5000 mg/kg body weight	3

2.4 Observation design

The treated animals were closely observed for incidence of mortality and clinical signs of intoxication periodically at one hour interval for 24 hours and twice daily for a period of 14 days.

3. RESULT AND DISCUSSION

3.1 Body weight

Wistar albino rats treated with the test compound **"MANGO SEED KERNEL FLOUR"** at the dose level of 5000 mg/kg body weight showed normal gain in body weight on day 7^{th} and 14^{th} (post treatment) presented in Table-3.

Table 3: Summary of body weight (gm)

Steps	Animal No.	Day 0	Day 7	% Gain/loss	Day 14	% Gain/loss
Step I: 5000 mg/kg b. wt	2019205 - 07	212	219	3.30	225	4.65
	2019205 - 08	208	215	3.36	228	9.61
	2019205 - 09	212	218	2.83	222	4.71

3.2 Clinical signs

The treated animals were closely observed for clinical signs of intoxication at every 1hour interval for 24 hours after dosing and thereafter twice a day for 14 days as presented in Table 4-a, b and c for all three steps i.e. 300, 2000 and 5000 mg/kg/body weight. All the rats were observed at least twice daily to observe any clinical signs or behavioral changes. These observations included changes in skin and fur, in the eyes and mucous membranes, respiratory, circulatory, central nervous and autonomic nervous systems, motor activity and behavioral changes. The following clinical signs were observed in rats to characterize the various systemic studies: Salivation, lacrimation, pale mucous membrane, diarrheal faeces, hunched posture, scratching, polyuria, hypoactivity etc. The clinical sign will be graded as 0 = Normal, + = Mild, ++= Moderate, +++ =High and ++++ = Severe.

Table: 4(a) Clinical signs and mortality

Step I: 300 mg/kg body weight **Sex: Female Rats**

Parameters	**Incidence of Clinical Signs Observed after Dosing on**																		**Mortality**
	Day 0					**DAY**													
	Min	Hour																	
	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
Clinical Signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table: 4(b) Clinical signs and mortality

Step II: 2000 mg/kg body weight **Sex: Female Rats**

Parameters	**Incidence of Clinical Signs Observed after Dosing on**																		**Mortality**
	Day 0					**DAY**													
	Min	Hour																	
	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
Clinical Signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table: 4(C) Clinical signs and mortality

Step III: 5000 mg/kg body weight Sex: Female Rats

Parameters	**Incidence of Clinical Signs Observed after Dosing on**																		**Mortality**
	Day 0					**DAY**													
	Min	Hour																	
	30	1	2	4	6	1	2	3	4	5	6	7	8	9	0	11	12	13	14	Total
Mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
Clinical Signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

3.3 Mortality

No mortality was recorded in any of the Wistar albino rats after administration of the test compound till the dose level of 5000 mg/kg body weight throughout the period of observation.

3.4 Necropsy findings

After sacrificing the mice, necropsy findings showed normal with no gross changes in abdominal cavity such as spleen, digestive system, liver and biliary ducts. Excretory system, adrenal and female genital organs showed normal color, consistency and no inflammatory changes up to highest tested dose level 5000 mg/kg body weight as shown in Table 5. Thoracic cavity was found to be normal without any fluid, mucous or blood etc. No changes were shown on lungs, heart. Thyroid and brain were normal in shape, size and surface up to 5000 mg/kg body weight dose level.

Table: 5 Summary of necropsy findings **Fate: Terminal Sacrifice**

S. No.	Finding	Female Rats		
		Dose (mg/kg)		
		300	2000	5000
1.	Terminal Sacrifice	3/3	3/3	3/3
2.	Abnormality detected	0/3	0/3	0/3
3.	Mortality	0/3	0/3	0/3

3.5 Discussion

On the basis of above findings, mango seed powder may practically be proven to be non-toxic after acute toxicity test. This is in accordance with a study by **Ashalatha et.al, (2015)** who concluded that it is to be safe to use mango seed kernel powder, as it didn't show toxicity till 5000 mg/kg body weight. It showed no mortality and histopathological changes with highest dose level in accordance with OECD guidelines. This study also indicated that mango seed powder is safe for longer use with dosage of 500 mg/kg body weight, as mango seed kernel powder possesses higher levels of phenolics and free radical scavenging activity. It is to be proven in a study that oral administration of mango seed powder help in reduction of elevated AST and ALT levels in Pb- acetate treated mice. Study done on mice (lead induced) has shown potential of mango by products to protect from oxidative stress, mitigate changes in hepatic biochemical and pathological parameters and genotoxicity of lead by **El Makawy et.al (2015).**

The study on swiss albino mice for anti diarrheal efficacy of mango seed kernel stated that the presence of secondary metabolites such as saponin, flavonoid, glycosides, tannin and alkaloids linked to antidiarrheal activity. Tannin helps in reduction of intestinal mobility, which is almost

15% in dried mango seed. Tannin served as astringent in cases of diarrhea, dysentery, urethritis etc **(Rajan et.al, 2012).**

Oyedeji et.al (2018) carried out a study on toxicity of mango seed kernel oil to clarify its potency. Due to limited knowledge related to toxicological status of mango seed kernel oil, its utilization has been very less. This study has pointed towards the fact that oils from mango seed were not toxic and were having good percentage of emulsion capacity, hence they are all safe with potential for domestic and commercial use.

As present study showed no sign of toxicity and mortality up to the highest dose level i.e. 5000 mg/kg body weight, it comes in category-5 according to ***Globally Harmonized System of Classification (GHS)***. It is considered when there is low toxicity; there is direct relevance for protection of animal / human health. A study carried out on aqueous extract of Irvingia gabonesis (African mango) acute toxicity revealed that it is non-toxic with oral LD_{50} greater than 5000mg/kg body weight, thus the low toxicity of Mango seed powder offers a wide range of safe beneficial uses as therapeutics by **Usman et.al (2019).**

4. CONCLUSION

Purpose of choosing higher dosage of powder was to establish the dose in accordance with OECD guidelines. As per the results obtained from present investigation, the mango seed powder in the dose up to 5g/kg didn't show any kind of variations in mice, so $1/10^{th}$ part i.e. 500 mg/kg body weight can be conveniently used for experimental study. According to globally harmonized classification system for chemical substances (GHS) category-5 (>5000 mg/kg) LD50 cut-off value of test compound is more than 5000 mg/kg body weight.

ACKNOWLEDGMENT

This work was facilitated at"***Toxicology Department,Institute for Industrial research &Toxicology"***F-209, Phase-1, U.P.S.I.D.C., MasuriDhaulana,Gulaothi Marg, Ghaziabad – 201302, Uttar Pradesh, India.

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