

## Pharmacological Evaluation of Excess Intake of *Amlarasa* (sour taste) with Special Reference to *Tamarindus Indica* Linn. Fruit Pulp.

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### ABSTRACT

**Introduction:** Ayurveda advocates balanced intake of all the *Rasa* (taste) in diet, which helps maintaining physiological health for excessive intake of any may produce undesired effects. *Amlarasa* (sour taste) is a liked and commonly consumed taste as an appetizer or as taste enhancer. Some people however are fond of it to the extent of excess consumption. Tamarind (*Tamarindus indica* Linn.) is a common source of sour taste in Indian food. Its pulp is used to make various food preparations and taste enhancers. **Materials and Methods:** The present study was planned to assess any adverse effect of *Amlarasa* (sour taste) having *Tamarindus indica* Linn. ripened fruit pulp as a representative, given in higher dose and for long duration to wistar albino rats. Therapeutically equivalent dose (TED) of 540 mg/kg and an overdose (TED×5 = 2700mg/kg) of tamarind fruit pulp, was administered orally for 60 days to Wistar Albino rats. Ponderal changes, gross behaviour, haematological, serum biochemical parameters were evaluated and histopathological study were carried out. **Results:** *Tamarindus indica* pulp (2700mg/kg) has shown mild fatty changes and cell infiltration in the liver sections. At therapeutic and higher dose level, blood sugar, serum uric acid and SGOT levels were found to be increased significantly. **Conclusion:** Study supports the concept of *Atiyoga* (over consumption) referring to *Amlarasa* lead to certain undesired health consequences.

**Keywords:** *Tamarindus indica* Linn., Sour taste, *Atiyoga*, Over consumption, *Amla rasa*.

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### Introduction

Ayurveda describes the importance of diet in the management of diseases and also highlights its importance in causing various diseases.<sup>[1]</sup> Each *Rasa* (taste) is recommended to be consumed in adequate proportion to maintain the health according to the body type, disease, season etc.<sup>[2]</sup> This is however seen that owing to the liking of a particular taste and due to culinary traditions of particular region, people are more prone to one taste over the other. *Amla* (sour) is a regular component of food as an appetizer or taste enhancer. Due to its pleasing taste, it is also consumed excessively by many as a routine dietary

habit. In southern part of India, dried tamarind is added to number of food preparations as a routine.<sup>[3]</sup> According to Ayurveda excessive intake of sour food leads to effects like dentine hypersensitivity, increased thirst, eye contraction, piloerection, aggravation of *kapha* and *pitta*, blood vitiation, muscular wasting, loosing compactness of body, oedema, suppuration of wounds, burns or fractures or swelling, burning sensation in throat, heart and chest etc.<sup>[4]</sup> Experimental or clinical studies have however not been conducted on effect of excessive intake of sour substances on biological systems. This study has firstly evaluated effects of excessive use (in terms of higher dose or long term use at normal dose) of sour taste with special reference to ripened fruit of *Tamarindus indica*

Linn. in Wistar Albino rats.

## Materials and methods

### Animals

Wistar strain Albino rats of either sex; weighing  $200 \pm 40$  g rats were used for the study. The animals were obtained from the animal house attached to Pharmacology laboratory of I.P.G.T & R.A, Gujarat Ayurved University. The animals were housed under standard laboratory conditions (temperature  $23 \pm 1^\circ\text{C}$ , relative humidity  $55 \pm 5\%$  and lighting 08:00-20:00 h). The institutional animal ethical committee approved the study protocol (Approval number: IAEC/9/11/14MD, 2010).

### Collection of *Tamarindus indica* Linn.

The ripened fruits of *T. indica* were collected from Morbi, Gujarat, in month of May. The sample was authenticated by pharmacognosist of the institute. Plant specimen was submitted at the pharmacognosy laboratory of the institute with voucher specimen no. Phm. 2010/11/6013 for future reference. Pulp of *T. indica* was separated from its seeds and preserved in air tight glass bottles.

### Dose fixation

Dose of fruit pulp of *T. indica* do not have a mention in any classical ayurvedic text. In *Aryabhisak*, 15 g pulp is reported to have a laxative effect.<sup>[5]</sup> API considers 4-10 g as its therapeutic dose range. Therefore, 6 g of tamarind pulp was considered as therapeutic dose for the experimental evaluation. The rat dose for experimental study was calculated by extrapolating the human dose to animal based on the body surface area ratio (based on table of Paget and Barnes).<sup>[6]</sup> The study was carried out with two dose levels, i.e., the therapeutically equivalent dose (TED) of 540 mg/kg and overdose i.e. TED $\times$ 5 (2700mg/kg). The fruit pulp of *T. indica* was mixed with distilled water and administered orally with the help of gastric catheter of suitable size sleeved on to a syringe nozzle.

## Experimental design

For evaluation of excessive use of *indica* fruit pulp, experiment was designed to evaluate effects of its long term use in regular and increased dose. Protocol was followed according to chronic toxicity of 60 days.<sup>[7]</sup>

The selected animals were divided into three groups of six animals each of either sex. First group received tap water besides regular food and served as control. The fruit pulp of *T. indica* at the dose of 540mg/kg and five times higher dose 2700mg/kg were administered to second and third groups respectively for 60 consecutive days. Body weight, food and water intake of all three groups were recorded every week during the whole study. Gross behaviour of all the animals was observed every 7<sup>th</sup> day according to claromarpurgo protocol.<sup>[8]</sup> On 60<sup>th</sup> day all animals were kept for overnight fasting. Next day blood was collected by supra-orbital puncture for estimation of hematological and serum biochemical parameters such as hemoglobin content, total and differential count of white blood cells, PCV, RBC and platelet count, MCV, MCH and MCHC, blood sugar,<sup>[9]</sup> serum cholesterol,<sup>[10]</sup> serum triglyceride,<sup>[11]</sup> HDL cholesterol,<sup>[12]</sup> blood urea,<sup>[13]</sup> serum creatinine,<sup>[14]</sup> serum glutamic pyruvic transaminase (SGPT),<sup>[15]</sup> serum glutamic oxaloacetic transaminase (SGOT),<sup>[16]</sup> serum total protein,<sup>[17]</sup> serum albumin and serum globulin,<sup>[18]</sup> serum alkaline phosphatase,<sup>[19]</sup> total bilirubin,<sup>[20]</sup> direct bilirubin,<sup>[21]</sup> uric acid<sup>[22]</sup> and serum calcium<sup>[23]</sup> estimated by using semi-autoanalyser. Animals were subsequently sacrificed by over dose of ether anaesthesia. The abdomen was opened through midline incision and important organs like liver, spleen, heart, kidney, thymus, uterus, ovary, testis, seminal vesicle and prostate were dissected. The extraneous tissues were removed and organs were weighed. Then they were transferred to bottles containing 10% formalin for the purpose of histopathological study.<sup>[24]</sup>

### Statistical analysis

The results are presented as Mean  $\pm$  SEM. The data generated during the study were subjected to one way

ANOVA with Dunnett's multiple's test as post-hoc test and the level of  $p < 0.05$  was set as significant.

## **Results**

Both the treated groups, at dose of 540mg/kg and at five folds higher dose (2700mg/kg) did not produce any mortality and other gross behavioural changes during entire duration of study except consistency of fecal pellets were somewhat gooey in higher dose treated group during first two weeks.

Body weight, food consumption and water intake is almost un-altered in both the doses of *T. indica* pulp administered groups (Table 1 to 3).

Blood sugar, serum uric acid and SGOT levels were significantly increased in *T. indica* pulp treated groups (Table 5). In haematological investigation, at the both dose levels, significant increase in total WBC count was found in comparison to control group. Significant decrease in serum triglyceride and urea is observed in *T. indica* (TED×5) treated group (Table 6).

High dose of *T. indica* pulp (TED×5) treated group showed mild fatty changes and cell infiltration in the liver sections (Figure 1). Sections of other organs showed normal cytoarchitecture. There was negligible ponderal changes found in wet organ weight (Table 4).

## **Discussion**

In both the treated groups, there were no significant changes found in gross behaviour, except mushy fecal pellets were observed in higher dose treated group during first week of drug administration. After one week, goeyness was gradually reduced and fecal output befitted normal. It may be due to higher dose of tamarind fruit pulp which is reported for possessing laxative activity.<sup>[25]</sup>

Body weight is a useful indicator of physical development

and health status of any living being. In both groups treated with *T. indica* normal progressive increase in body weight similar to that of control group was found to be indicative of no degenerative pathology occurring during tamarind administration. *Tamarind* pulp contains 15.8 – 25.0% invert sugar and so a consequently rise of blood sugar level in treated groups seems natural.<sup>[26]</sup>

SGOT (AST) and SGPT (ALT) are indicative of liver functions and are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute haemolytic anaemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.<sup>[27]</sup> In present study, significant increase was observed in SGOT/AST activity in both the doses of *T. indica* pulp treated groups. This advocated that liver enzymatic stimulation may occur in treated groups.

Uric acid is a strong reducing agent and a potent antioxidant. In humans, over half the antioxidant capacity of blood plasma comes from uric acid.<sup>[28]</sup> Significant increase was observed in serum uric acid in *T. indica* treated groups. It has been reported that high intake of dietary purine as well as fructose can cause increased levels of uric acid.<sup>[29]</sup> Moreover, Tamarind fruits also exhibits high antioxidant capacity due to its high phenolic content.<sup>[30]</sup>

In this study, significant lowering in serum triglyceride and decrease in serum cholesterol and maintained HDL level was found. It is therefore safe for heart patient and normal individuals. Martinello et al. showed that *T. indica*

fruit extract decreases serum total cholesterol, triglyceride and LDL.<sup>[31]</sup>

Haematological parameters did not show any alteration except increased in WBC counts after 60 days administration. Increased WBC count is indicative of inflammatory conditions of certain organs. This shows that the drug is having such property of causing inflammatory changes in the body on chronic administration. Histopathological sections of liver (plate 1) from higher dose group showed micro fatty changes in liver. Hence it may be one of the reasons behind observed increase in total WBC count. It is important to note that, tamarind at both the doses, did not produce any pathological changes in organs like heart, kidney spleen, stomach, intestine, testis and ovary.

Results provided that haematological and serum parameters such as WBC count, serum uric acid and SGOT levels were significantly increased in both *T indica* pulp treated groups. This indicates that not only higher dose but normal dose being administered for long duration may also lead to the untoward effects. Thus the concept of 'atiyoga' is not limited to the higher dose alone, but also includes long term consumption at a normal dose.

In the study, after consideration of various aspects such as frequent use of the plants as a diet and drug, approval of animal ethics committee, duration of the study, other

limited resources etc, tamarind fruit was taken as a representative among many *amla rasa* predominant plants materials. It has however been proposed to carry out similar studies with more *amla rasa* predominant plants for further validation of concept of *atiyoga sevana* prescribed in Ayurvedic classical texts.

### Conclusion

Experimental evaluation of *T. indica* on Wistar Albino rats at 540 mg/kg (therapeutic dose) and 2700 mg/kg (higher dose) administration altered the haematological and biochemical indicators such as increase in total WBC count, blood sugar, serum uric acid and SGOT; and decrease in serum triglyceride and urea. It also showed changes in liver cyto-architecture at high dose. This result indicated that excessive and continuous use of *Tamarindus indica* Linn. fruit pulp for long term ( 60 days ) may cause biochemical alterations. As this is a preliminary attempt to evaluate effects of excessive intake of sour tasting food with example of *Tamarindus indica* Linn., further observational studies such as case control or cohort may be required to establish the causal relationship between excessive use (in terms of higher dose or long term use at normal dose) of sour taste and subsequent untoward effects mentioned in Ayurvedic texts.

Table –1: Effect of *Tamarindus indica* Linn.on body weight

Parameters	Control(g)	<i>T. indica</i> (TED)	% change in comparison to control	<i>T. indica</i> (TED×5)	% change in comparison to control
Body weight(g)	12.00 ± 4.68	15.83 ± 6.28	31.91↑	12.83 ± 8.13↑	6.91↑

Data: Mean ± SEM

**Table – 2 Effect of *Tamarindus indica* Linn. on food consumption**

Days	Control(g)	<i>T. indica</i> (TED)	<i>T. indica</i> (TED×5)
1 <sup>st</sup> week	14.941 ± 0.811	13.728 ± 1.381	14.050 ± 1.468
2 <sup>nd</sup> week	15.454 ± 0.758	13.886 ± 0.779	14.067 ± 0.944
3 <sup>rd</sup> week	16.634 ± 1.130	12.794 ± 0.979	12.777 ± 1.031
4 <sup>th</sup> week	15.146 ± 0.774	13.945 ± 0.764	13.864 ± 0.884
5 <sup>th</sup> week	15.158 ± 0.735	14.415 ± 1.215	14.443 ± 1.395
6 <sup>th</sup> week	14.661 ± 0.858	14.162 ± 0.820	14.267 ± 1.046
7 <sup>th</sup> week	18.579 ± 1.097	13.491 ± 1.070	14.417 ± 1.158
8 <sup>th</sup> week	18.178 ± 0.509	12.435 ± 0.959	12.517 ± 1.115
Terminal	17.449 ± 0.639	16.495 ± 1.335	16.728 ± 1.502

Data: Mean ± SEM

**Table – 3 Effect of *Tamarindus indica* Linn. on water intake**

Days	Control(ml/100g body weight)	<i>T. indica</i> (TED) (ml/100g)	<i>T. indica</i> (TED×5) (ml/100g)
1 <sup>st</sup> week	14.508 ± 0.779	12.738 ± 1.135	13.040 ± 1.231
2 <sup>nd</sup> week	13.035 ± 0.958	12.397 ± 0.903	12.555 ± 1.010
3 <sup>rd</sup> week	14.372 ± 0.875	12.753 ± 1.887	12.724 ± 1.883
4 <sup>th</sup> week	14.246 ± 0.728	12.201 ± 1.128	12.132 ± 1.180
5 <sup>th</sup> week	14.090 ± 0.749	11.553 ± 1.306	12.451 ± 1.398
6 <sup>th</sup> week	15.383 ± 0.737	13.134 ± 1.083	13.247 ± 1.263
7 <sup>th</sup> week	13.319 ± 0.313	11.988 ± 1.294	12.107 ± 1.439
8 <sup>th</sup> week	13.193 ± 0.916	10.579 ± 0.983	10.659 ± 1.111
Terminal	15.189 ± 1.033	11.721 ± 0.414	11.390 ± 0.623

Data: Mean ± SEM

Table –4 Effect of *Tamarindus indica* Linn.on ponderal changes

Parameters	Control(g/100g)	<i>T. indica</i> (TED)	% change in comparison to control	<i>T. indica</i> (TED×5)	% change in comparison to control
Liver(g)	2.780 ± 0.090	2.790 ± 0.070	00.35	2.750 ± 0.120	01.07
Spleen(mg)	0.200 ± 0.010	0.180 ± 0.012	10.00	0.209 ± 0.015	04.50
Kidney(mg)	0.720 ± 0.024	0.746 ± 0.042	03.61	0.723 ± 0.017	00.41
Thymus(mg)	0.118 ± 0.008	0.128 ± 0.002	08.47	0.136 ± 0.005	15.25
Heart(mg)	0.306 ± 0.013	0.298 ± 0.015	02.61	0.312 ± 0.009	01.96

Data: Mean ± SEM

Table – 5 Effect of *Tamarindus indica* Linn. on various serum biochemical parameters

Parameters	Control	<i>T. indica</i> (TED)	% change in comparison to control	<i>T. indica</i> (TED×5)	% change in comparison to control
Blood sugar (mg/dl)	100.75 ± 4.29	105.33 ± 4.67	0.45	128.33 ± 8.96**	27.37
Total cholesterol (mg/dl)	63.50 ± 3.87	61.83 ± 5.99	2.62	57.00 ± 2.07	10.23
Triglyceride (mg/dl)	102.50 ± 5.02	87.33 ± 6.48	14.8	60.00 ± 3.33***	41.41
HDL (mg/dl)	41.12 ± 3.99	41.83 ± 4.28	0.43	41.00 ± 2.02	0.29
Blood urea (mg/dl)	91.13 ± 4.96	83.33 ± 6.87	8.55	63.17 ± 4.08***	30.68
Serum creatinine (mg/dl)	0.60 ± 0.04	0.65 ± 0.04	8.33	0.58 ± 0.03	3.33
SGPT (IU/L)	72.00 ± 07.58	82.17 ± 11.16	14.12	75.17 ± 05.00	4.40
SGOT (IU/L)	140.12 ± 6.37	212.67 ± 18.89**	51.77	192.67 ± 15.50**	37.50
Total protein (g/dl)	7.40 ± 0.16	7.87 ± 0.09	6.35	7.60 ± 0.21	0.02
Albumin (g/dl)	3.75 ± 0.11	3.68 ± 0.12	1.86	3.85 ± 0.13	2.66
Globulin (g/dl)	3.52 ± 0.14	4.18 ± 0.14	18.75	3.75 ± 0.21	6.53
Alkaline phosphatase (IU/L)	277.50 ± 34.19	215.83 ± 25.11	22.22	209.00 ± 39.22	24.68
Total bilirubin (mg/dl)	0.50 ± 0.04	0.43 ± 0.03	14	0.45 ± 0.06	10
Direct bilirubin(mg/dl)	0.19 ± 0.01	0.12 ± 0.02	36.84	0.15 ± 0.03	21.05
Uric acid (mg/dl)	0.79 ± 0.13	1.40 ± 0.15**	77.21	1.32 ± 0.38	67.08
Serum calcium (mg/dl)	8.65 ± 0.39	8.17 ± 0.25	5.54	8.57 ± 0.16	0.92

Data: Mean ± SEM, \*\*P&lt;0.01, \*\*\*P&lt;0.001 (comparison to control group, unpaired t test)

Table – 6 Effect of *Tamarindus indica* Linn. on various haematological parameters

Parameters	Control	<i>T. indica</i> (TED)	% change in comparison to control	<i>T. indica</i> (TED×5)	% change in comparison to control
WBC (10 <sup>3</sup> /Cumm)	7525.00±874.59	10416.67±378.96*	38.42	10383.33±2077.08	37.98
Neutrophil (%)	19.25 ± 03.36	18.17 ± 04.97	5.61	18.33 ± 04.48	4.77
Lymphocyte (%)	75.75 ± 03.47	76.00 ± 04.99	0.33	75.83 ± 04.57	0.10
Eosinophil (%)	02.63 ± 00.32	03.17± 00.40	20.53	03.00 ± 00.37	14.06
Monocyte (%)	02.38 ± 00.26	02.67 ± 00.42	12.18	02.83 ± 00.31	18.90
Hb(g/dl)	15.69 ± 00.30	16.35 ± 00.33	4.20	16.25 ± 00.26	3.56
PCV (%)	49.48 ± 00.10	52.03 ± 00.90	5.15	51.35 ± 00.83	3.77
RBC (10 <sup>6</sup> /cu mm)	08.85 ± 00.19	09.11 ± 00.24	2.93	09.02 ± 00.20	1.92
MCV (fl)	55.84 ± 00.37	57.17 ± 00.81	2.38	56.97 ± 00.77	2.02
MCH (pg)	17.80 ± 00.16	17.97 ± 00.35	0.95	18.03 ± 00.36	1.29
MCHC (g/dl)	31.78 ± 00.25	31.33 ± 00.34	1.41	31.65 ± 00.23	0.40

Data: Mean ± SEM, \*P<0.05, \*\*P<0.001 (comparison to control group, unpaired t test)

Figure -1. Effect of *T. indica* fruit pulp on cytoarchitecture of Liver

Plate - 1: Photomicrographs of Liver

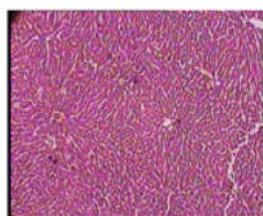


Fig - 1a  
Normal Control group (1×100)

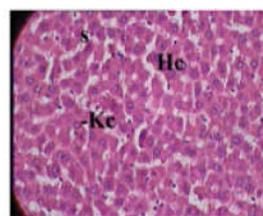


Fig - 1b  
Normal Control group(1×400)  
He-Hepatic Cell;  
Kc-Kupffer Cell; S-Sinusoid

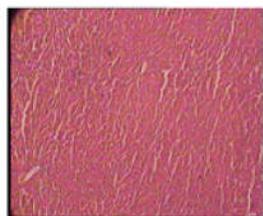


Fig - 1c  
Amlika TED treated group (1×100)

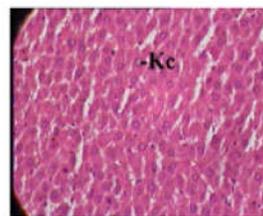
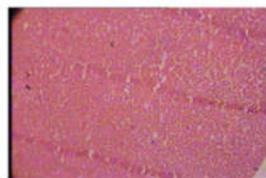
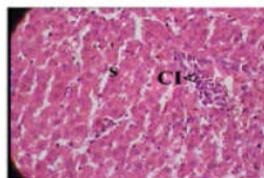


Fig - 1d  
Amlika TED treated group (1×400)  
Kc-Kupffer Cell



**Fig - 1e**  
Amlika TEDx5 treated group  
(1x100)



**Fig - 1f**  
Amlika TEDx5 treated group  
(1x400)  
CI- Cell infiltration, S-Sinusoid

TED- Therapeutic equivalent dose

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