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Pharmacological Study

Evaluation of acute toxicity and anti-ulcerogenic study of rhizome starch of two source plants of *Tugaksheeree* (*Curcuma angustifolia* Roxb. and *Maranta arundinacea* Linn.)

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Abstract

Background: Disorders like hyperacidity and gastric ulcers are found very frequently now days because of a faulty lifestyle. Starches (*Satwa*) obtained from the rhizomes of two plants namely, *Curcuma angustifolia* Roxb. (Fam. Zingiberaceae) and *Maranta arundinacea* Linn. (Fam. Marantaceae) are used in folklore practice, as *Tugaksheeree,* for the treatment of the above-mentioned complaints. **Aim:** To assess the acute toxicity potential of the *C.angustifolia* and *M. arundinacea* along with their assessment for adaptogenic activity, by noting their effect on forced swimming-induced hypothermia and gastric ulceration in rats. **Materials and Methods:** For acute toxicity study, the effect of test drugs *C. angustifolia* and *M. arundinacea* rhizome starch were studied after a single administration of up to three dose levels, with 4400 mg/kg as the maximum dose. The animals were observed for 72 hours periodically and mortality was recorded up to seven days. The adaptogenic and anti-ulcer activities were assessed by determining and comparing the changes in rectal temperature, ponderal changes, ulcer index and histopathological parameters in the test drug group with that of stress control group. **Results:** Both the drugs did not produce any toxic symptoms or mortality even up to the maximum dose level of 4400 mg/kg. Both the test drugs significantly reversed the stress-induced gastric ulceration in comparison to stress-control rats. Starch from rhizome of *C. angustifolia* reversed forced swimming-induced hypothermia apparently, but not to a significant extent. However, the reversal of hypothermia found statistically significant in the rhizome starch of the *M. arundinacea* treated group. **Conclusion:** *M. arundinacea* had better anti-stress activity in comparision to *C. angustifolia*.

Key words: *Curcuma Angustifolia* Roxb., Gastric Ulcer, *Maranta Arundinacea* Linn., Starch, Swimming, *Tugaksheeree*

Introduction

Tugaksheeree is one among the important ingredients in many medicinal formulations like *Chyavanaprasha*,[1] *Pippalyadyavaleha*,[2] *Narikela khanda paka*,[3] etc., Starch obtained from the rhizomes of two plants, namely, *Curcuma angustifolia* Roxb. (Fam. Zingiberaceae) and *Maranta arundinacea* Linn. (Fam. Marantaceae) are used as *Tugaksheeree*.[4-8] They are used in folklore practice in conditions like hyperacidity and also as a nutritional food supplement. To date, no scientific data is available on the efficacy and safety of these drugs. Hence, the present study has been undertaken to assess the acute toxicity potential of the test plants along with their assessment for adaptogenic activity, by noting their effect on forced swimming-induced hypothermia and gastric ulceration.

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Materials and Methods

Procurement of test drugs

The starches from both the plants were collected by a scholar from two places. The rhizomes and starches of *C. angustifolia* were collected from the Purva Mandala District of Madhya Pradesh and those of *M. arundinacea* were collected from Dakshina Kannada District of Karnataka, and authenticated. A voucher specimen was deposited in the Pharmacognosy Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar.

Animals

Wistar strain albino rats of both sexes weighing 180 to 260 g were used in the experimental study. The animals were obtained from the Animal House attached to the Pharmacology Laboratory, IPGT and RA, Jamnagar. Animals were housed at 22 ± 2°C with constant humidity 50 – 60%, on a 12-hour natural day and night cycle. Animals were fed with diet - Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water *ad libitum*. All the experiments were carried out after obtaining approval from the Institutional Animal Ethics Committee (IAEC) (IAEC/07-08/01/01; Dt. 01.09.2006).

Dose fixation and schedule

The human dose of both the test drugs was 12 g per day. Considering the adult human dose of both *C. angustifolia* and *M. arundinacea* starch, the dose (Therapeutic Equivalent Dose - TED) for the experimental study was calculated by converting the human dose to an animal dose, based on the body surface area ratio, using the table of Paget and Barnes,[9] which was 1100 mg/kg body weight of a rat. The stock solution was freshly prepared using an adequate quantity of water, with both the starch samples, and used for all experimental purposesThe test drugs were administered according to the body weight of the animals by the oral route with the help of a No. 6 gastric catheter sleeved onto a syringe.

Acute toxicity with gross behavior

This refers to the immediate harmful effects generated by a single dose of drug, several levels higher than the therapeutically equivalent dose (TED).[10]

Total 32 animals of both sexes weighing 180-260 g were taken and divided into 8 groups, each group comprised of 4 animals. In the acute toxicity study three dose levels were studied that is TED × 2, TED × 3, and TED × 4, so both the test drugs were given up to 4.4 g/kg for a higher dose level. First group served as a control group and received only tap water, second test drug group with dose TED × 2, third with dose TED × 3, and fourth with dose TED × 4 for both the test drugs [Table 1]. During the Acute toxicity studies, the animals were observed for general appearance, cage-side behavior, including increased or decreased motor activity were studied as per standard protocol.

Adaptogenic and gastro-cytoprotective activity

A stress-induced hypothermia test was performed as described in previous research study.[11] Eighteen rats weighing between 220 and 240 g were selected for the study and divided into three groups of six animals each and namely Group C, I and II. Group-C animals received only tap water and served as the stress control group. Group-I and Group-II animals were administered test drug starch of *C. angustifolia* and *M. arundinacea* suspended in water, at dose of 1100 mg/kg, for 7 consecutive days respectively.

The rats were kept in individual metabolic cages, to prevent coprophagy, and fasted for 12 hours with access to water *ad libitum*. On the seventh day, one hour after drug administration, the initial rectal temperature of individual rats was noted. After noting the initial rectal temperature, the rats were kept inside specially arranged containers, which were made up of plexiglass with holed lids. The water level was maintained up to a 25cm height and the temperature of water was maintained at 25 ± 2°C. The rats were placed in the container and exactly after 20 minutes of exposure to the stressed condition, the rats were taken out individually and the final rectal temperature of each rat was noted.

The stress-induced ulcer test was carried out by the method described in previous research study[12] and modified according to experimental needs. The rats after noting their final rectal temperature were again exposed to a swimming stress inside the same container. At the end of a 14-hour period the body weight of each rat was noted and rats were sacrificed by cervical dislocation. The stomach was then dissected and opened along the greater curvature and the inner surface gently washed and examined with a magnifying lens for ulceration in the glandular area as well as in the rumen (if any), for determining the severity of the ulcer and calculating the ulcer index.[13] Part of the ulcerated area was selected for histological examination following the standard protocols.

Statistical analysis

All the values were expressed as mean ± SEM (Standard Error of Mean). The data was analyzed by an unpaired '*t*' test. A level of $P < 0.05$ was considered as statistically significant

Results

Acute toxicity with gross behavior

No mortality was observed in both the test drug–administered groups up to seven days and also no toxic signs and symptoms were recorded. In the gross behavior assessment no significant changes could be observed.

Anti-ulcer study

In the stress control group a decrease in body weight was observed in all the rats, in a uniform manner. However, in both the treated groups, 50% of the animals gained body weight,

Table 1: Grouping for acute toxicity study

Group No.	Drug	Dose (mg/kg)
1	Control (Tap water)	Q.S.
2	CAS - I	2200
3	CAS - II	3300
4	CAS - III	4400
5	Control (Tap water)	Q.S.
6	MAS - I	2200
7	MAS - II	3300
8	MAS - III	4400

C.A.S.: Starch of *Curcuma angustifolia* R, M.A.S.: Starch of *Maranta arundinacea* L

whereas, 50% of the animals lost body weight. The magnitude of observed change in body weight in both the test drug treated groups was also similar. When the observed mean change in the body weight was compared with the stress control group, a marginal gain was observed in both the groups, which was statistically insignificant [Table 2].

An apparent decrease in rectal temperature was observed in both the treated groups in comparison to the stress control group, however, the observed decrease was statistically insignificant [Table 3].

An apparent and statistically highly significant decrease in ulcer index was observed in both the test drug administered groups in comparison to the stress control rats [Table 4]. The histological examination also showed a significant attenuation of intensity of the stress-induced ulcer [Figures 1-4].

Discussion

The starch obtained from the rhizomes of two plants, *C. angustifolia* and *M. arundinacea*, are used as *Tugaksheeree*, one of the important medicines. It is used in folklore practice for treating *Amlapitta* and also as a nutritional food supplement in the Dakshina Kannada district of Karnataka. There is not much experimental data available on these drugs, especially related to their safety profile. The drug, whenever it is administered to a biological system, may produce different types of interactions and dose-related responses. In most of the cases these responses are desired and useful, but there are a number of other adverse effects that may also be produced. Considering these points, a study on acute toxicity with gross behavior was planned.

In the acute toxicity study the effect of test drug was studied after a single administration up to TED × 4 (4400 mg/kg), as the maximum dose. The animals were observed for 72 hours periodically and mortality was recorded up to seven days. Both the drugs did not produce any toxic symptoms or mortality even up to the maximum dose level of 4400 mg/kg. The LD_{50} value was likely to be very much higher than the maximum dose used in this study. This clearly indicated that the test drugs, in the given form, were not likely to have any toxicity potential at the dose level used therapeutically.

An ulcer is characterized by the disruption of mucosal integrity leading to a local defect or excavation due to active inflammation. Gastric ulcer, being the most prevalent gastrointestinal disorder, is considered as a major therapeutic target. The pathophysiology of an ulcer is considered to be due to an imbalance between aggressive factors (acid, pepsin, *H.Pylori*, and non-steroidal anti-inflammatory drugs [NSAIDs]) and local mucosal defensive factors (mucous bicarbonate, blood flow, and prostaglandins). The integrity of the gastroduodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors.[14]

Stress ulcers are due to both physiological and psychological factors (which are crucial for gastrointestinal defense) and increased accumulation of acid and pepsin, leading to auto-digestion of the gastric mucosa.[15]

Moreover, stress-induced ulcers can be prevented partially or entirely by vagotomy, and vagal overactivity has been suggested to be the principal factor in stress-induced ulceration.

Forced swimming of the animals for up to 14 hrs to induce the stress leads to decrease in body weight to a great extent, due to utilization of a large amount of energy by the body — this is what the stress control animals showed. The starch of both the test drugs non-significantly reversed this. The reason for this may be the nutritional property of the test drugs or the adaptogenetic effect.

Subjecting rats to forced swimming stress for a short duration (20 minutes in the present study) causes a marked decrease in the rectal temperature. This hypothermia is reversed by drugs possessing significant anti-stress activity. Stress-induced hypothermia is a very powerful stimulus, which is not easily reversed. If reversed, it is suggestive of the presence

Table 2: Effect of *Curcuma angustifolia* R. and *Maranta arundinacea* L. starch on the body weight of a swimming stress-induced gastric ulcer in rats

Group	Dose (mg/kg)	Initial body weight	Final body weight	Actual change
Stress control	Q.S.	236.00±12.08	216.00±12.08	–20.00±03.16
*C.A. (*n* - 3)	1100	240.00±30.75	273.33±33.33	+33.66±06.83
#C.A. (*n* - 3)	1100	263.33±27.28	236.66±23.33	–26.67±06.67
*M.A. (*n* - 3)	1100	233.00±18.55	256.67±21.85	+ 33.33±06.67
#M.A. (*n* - 3)	1100	263.33±14.52	220.00±15.27	–43.33±12.00

C.A.: *Curcuma angustifolia* R, M.A.: *Maranta arundinacea* L, Data: Mean±SEM (*three animals from both the test drug groups gained weight, #three animals from both the test drug groups lost weight, hence, the actual change in the body weight was calculated separately)

Table 3: Effect of *Curcuma angustifolia* R. and *Maranta arundinacea* L. starch on rectal temperature in swimming stress-induced gastric ulcer in rats

Group	Dose (mg/kg)	Initial rectal temperature	Final rectal temperature	Actual change	% Change	% Change in rectal temperature	% Change
Stress control	Q.S.	38.33±00.12	32.13±00.34	06.87±00.19	-	16.16±00.70↓	-
C.A.	1100	38.15±00.16	32.51±00.47	05.42±00.63	21.10↓	14.70±01.47↓	09.03↓
M.A.	1100	38.58±00.09	33.05±00.40	05.48±00.36	20.23↓	14.21±00.44↓	12.06↓

C.A.: *Curcuma angustifolia* R, M.A.: *Maranta arundinacea* L, Data: Mean±SEM, ↓: Decrease

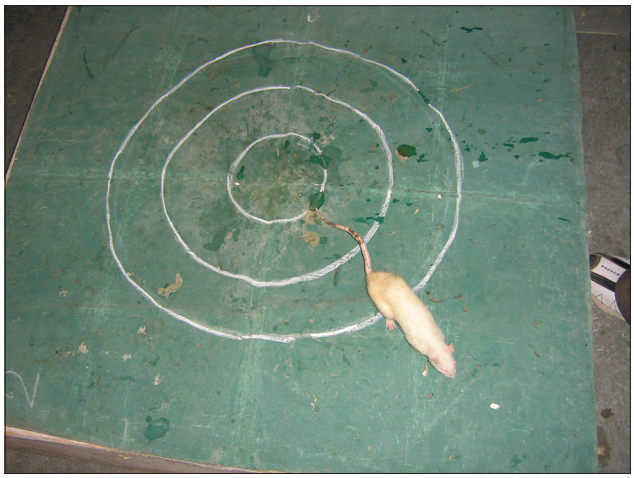

Figure 1: Photograph showing acute toxicity with gross behavior

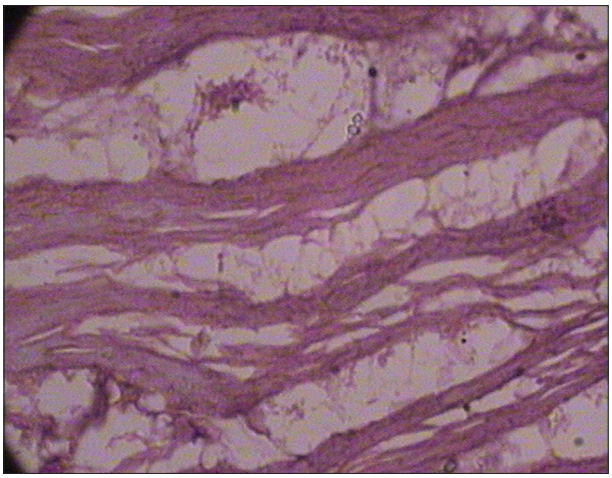

Figure 2: Photomicrograph of the representative sections of the stomach of albino rats from swimming stress, control group (I X 400 magnification). Note: Massive destruction of the epithelial layer with hemorrhagic patches, submucosal edema

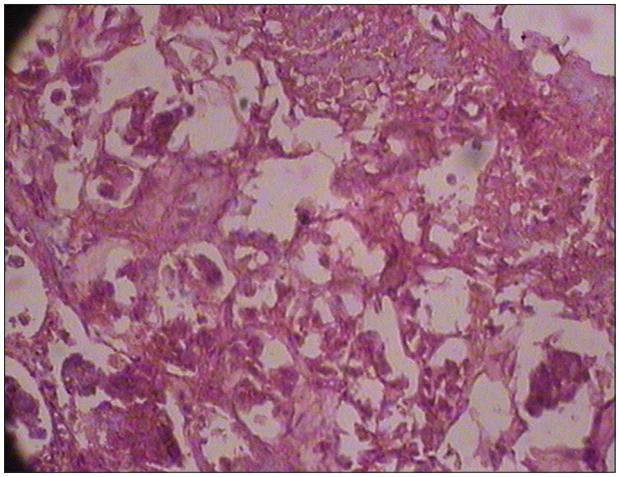

Figure 3: Photomicrograph of representative sections of the stomach of albino rats of the *Curcuma angustifolia* Roxb.–treated group (I X 400 magnification). Note: Comparatively less epithelial layer damage

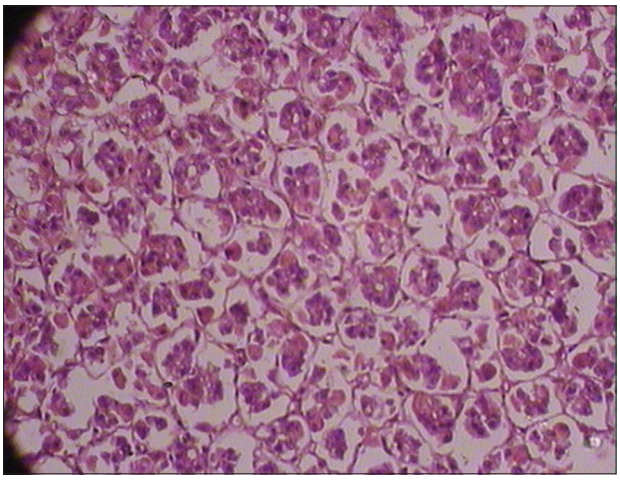

Figure 4: Photomicrograph of representative sections of the stomach of albino rats of the *Maranta arundinacea* Linn.–treated group (I X 400 magnification). Note: Almost normal cyto-architecture

Table 4: Effect of *Curcuma angustifolia* R. and *Maranta arundinacea* L. starch on the ulcer index in swimming stress-induced gastric ulcer in rats

Group	Dose (mg/kg)	Ulcer index (in mm)	% Change
Stress control	Q.S.	03.30±0.51	-
C.A.	1100	01.23±00.17**	37.27↓
M.A.	1100	01.15±00.54	65.15↓

C.A.: *Curcuma angustifolia* R, M.A.: *Maranta arundinacea* L, Data: Mean±SEM, ↓: Decrease, *P<0.01, **P<0.05

of significant anti-stress activity. In the present study starch from *C. angustifolia* has apparently reversed forced swimming induced hypothermia, but not to a significant extent. However, the reversal of hypothermia has been statistically significant in the group of rats treated with starch from *M. arundinacea*. This suggests that, *M. arundinacea* has better anti-stress activity.

The stress-mediated free radical generation and acid stimulation may lead to gastric ulceration. In the present study, both the test drugs significantly reversed stress-induced gastric ulceration. The protective action of starch from both the test drugs against stress-induced ulceration could be due to its histamine antagonistic, anti-secretory, and anti-cholinergic effects. The other possible mechanism of prevention for stress-induced ulcer could be a decrease in the lipid peroxidation and enhancement of the glutathione content in the stomach.

Analysis of the data generated as a whole indicates that, the starch samples obtained from both *C. angustifolia* and *M. arundinacea* possess good anti-ulcer activity against stress-induced ulcers. *M. arundinacea* has a better activity profile. Furthermore, as it reverses stress hypothermia besides attenuating stress ulcer, it seems to have better anti-stress effects. The exact mechanisms involved in reducing the stress ulcer are yet to be elucidated. The possibilities are: Reduction in the generation of free radicals or enhancement and strengthening of the body's anti-oxidant systems, like increasing

the activity of catalase and superoxide dismutase (SOD), or increasing the glutathione of the tissue.

As already been mentioned, *Tugaksheeree* is one among the important ingredients in many medicinal formulations like *Chyavanaprasha*, *Pippalyadyavaleha*, *Dadimashtaka Churna*, *Talisadichurna*, and so on. Starch obtained from the rhizomes of two plants, *C. angustifolia* and *M. arundinacea*, are used as *Tugaksheeree*. They are used in folklore practice (certain tribes of Dakshina Kannada district of Karnataka) for the disease *Amlapitta* and also as a nutritional food supplement. The data generated during the present study provides strong and unequivocal pharmacological evidence to the presence of the desired activity in both the candidate plants. Either of them can be used as *Tugaksheeree* based on the cost and availability. If there is no difference in these aspects, then *M. arundinacea* is preferred.

Conclusion

Both the drugs *C. angustifolia* and *M. arundinacea* were non-toxic up to the maximum dose level of 4400 mg/kg, as revealed by the acute toxicity with gross behavior test. The starch of *M. arundinacea* has better adaptogenic activity than the starch of *C. angustifolia*.

References

1. Agnivesha, Charaka, Dridhabala, Charaka Samhita, Chikitsa Sthana, Adhyaya,1-1/68, edited by Yadavji Trikamji Acharya, Reprint ed. Chaukhambha Surabharati Prakashan, Varanasi, 2009; 381.
2. Vangasena, Vangasena Samhita, Amlapittarogadhikara, 59/84, edited by Roy RK. Pracchya Prakashan, Varanasi, 2010; 524.
3. Anonymous, Yogaratanakara, Uttarardha, Amlapitta Chikitsa, 5/1-2, commentary by Vaidya Laxmipati Shastri and Brahma Shankar Sastri, reprint ed. Chaukhambha Surabhararathi Prakashan, Varanasi, 2008; 242.
4. Sharma PV. Dravyaguna Vijnana, Part-II. reprint ed. Varanasi: Chaukhambha Bharati Academy; 2009. p. 612.
5. Ibidem. Dravyaguna Vijnana, Part-II. p. 742.
6. Vaidya BG. Nighatu Adrasha. Vol.- 2. reprint ed. Varanasi: Chaukhambha Bharati Academy; 2009. p. 558.
7. Ibidem. Nighatu Adrasha. Vol.- 2. p. 579-80.
8. Lucas DS. Dravyaguna Vijnana. Vol. 2. 1st ed. Varanasi: Chaukhambha Vishwabharathi; 2008. p. 672-3.
9. Paget GE, Barnes JM. Evaluation of drug activities. Pharmacometrics. In: Lawranle DR, Bacharch AL, editors. Vol. 1. New York: Academic Press; 1969.
10. Guidance document on acute oral toxicity testing. OECD Environment, Health and Safety Publications Series on Testing and Assessment. Available from: http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd24.pdf.
11. Sheth UK. Selected topics in experimental pharmacology. Bombay: The Kothari Book Depot; 1972. p. 137.
12. Parmar NS, Jagruti KD. A review of the current methodology for the gastric and duodenal anti-ulcer agents. Indian J Pharmacol 1993;25:120-35.
13. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd ed. Delhi: Vallabh Prakashan; 1999. p. 148-50.
14. Dhamani P, Vijay Kumar K, Srivastava S, Palit G. Ulcer healing effect of Anti-Ulcer Agents: A Comparative Study. The Internet Journal Of Academic Physician Assistants 2003;3 (2):1-4.
15. Goel RK, Bhattacharya SK, Gastroduodenal mucosal defense and mucosal protective agents. Indian J Exp Biol 1991;29:701-14.

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हिन्दी सारांश

तुगाक्षीरी(कुर्कुमा अन्गुस्टिफ़ोलिया एवं मराण्टा अरुण्डिनेसिया) का संभाव्य विष प्रभाव और अम्लपित्तजन्य व्रण पर प्रभाव का चूहों मे अध्ययन

एन. राजशेखर, बी.के. अशोक, परमेश्वर पी. शर्मा, बी. रविशंकर

तुगाक्षीरी के नाम से दो वनस्पतियों (कुर्कुमा अन्गुस्टिफ़ोलिया एवं मराण्टा अरुण्डिनेसिया) का कन्द सत्व अम्लपित्त रोग मे उपयोग करते है। प्रस्तुत अध्ययन मे दोनो द्रव्यों का चूहों मे संभाव्य विष प्रभाव और अम्लपित्तजन्य व्रण पर होनेवाले प्रभाव का स्विमिंग स्ट्रेस इण्यूसड अल्सर मे प्रायोगिक अध्ययन किया गया। इस अध्ययन मे दोनों द्रव्यों का कोई विष प्रभाव नही पाया गया। अम्लपित्तजन्य व्रण को रोकने कि क्षमता दोनो द्रव्यों मे पायी गयी और दोनों मे से मराण्टा अरुण्डिनेसिया का प्रभाव कुर्कुमा अन्गुस्टिफ़ोलिया से थोडा ज्यादा पाया गया।