

Early Human Safety Study of Turmeric Oil (*Curcuma longa* Oil) Administered Orally in Healthy Volunteers

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Abstract

Objective : Turmeric extract and turmeric oil have shown chemoprotective effect against chemically-induced malignancies in experimental animals. They can reverse precancerous changes in oral submucous fibrosis in humans. The use of turmeric or *Curcuma longa* Linn as a spice and household remedy has been known to be safe for centuries. In view of the long term administration required for cancer prevention a Phase I clinical trial of turmeric oil (TO) was designed to study the safety and tolerance of TO in volunteers for a period of 3 months.

Material and Methods : Nine healthy volunteers between 20 and 33 years of age were tested for haemoglobin, blood counts, liver and kidney functions, bleeding and clotting time and serum electrolytes initially and at 1 and 3 months of treatment. They were administered 0.6 ml of TO three times a day for 1 month and 1 ml in 3 divided doses for 2 months. The acute tolerability study on Day 1 was conducted in a Clinical Pharmacology daycare Unit. Blood pressure and pulse were recorded frequently on Day 1 and at 24, 48, 72 and 96 hours and fortnightly till 12 weeks. Volunteers were daily supervised for TO intake as well as for any side effects throughout the study period.

Results : Nine volunteers were enrolled for the study. One discontinued on 3rd day for allergic skin rashes which, on discontinuation of TO, gradually disappeared by two weeks. Another discontinued on 7th day for intercurrent fever requiring antibiotic treatment. Seven volunteers completed the study. There was no effect of TO, in two doses, on pulse and blood pressure and no side effects in acute tolerability study on Day 1. There was no effect of TO intake on weight, blood pressure, symptoms and signs upto 12 weeks.

There was no clinical, haematological, renal or hepatic-toxicity of TO at 1 month and 3 months. Serum lipids did not show significant change except in one volunteer (reversible).

Conclusions : In view of the potential for reversing oral submucous fibrosis, a precancerous condition for oral cancer, TO, can be recommended directly for a Phase II trial in patients.

INTRODUCTION

Haridra or *Curcuma longa* Linn, commonly known as turmeric has been used for centuries as a spice and household remedy. In Ayurveda its use has also been recommended for various medical indications like wound healing, nausea, indigestion, inflammation, liver diseases, improving skin complexion etc.¹⁻³ Traditional use of turmeric

is also described in several other countries.^{4,5} Nagabhushan and Bhide in 1987 observed the anticancer activity of turmeric in benzo(a)pyrene (BP)-induced gastric tumours in mice.⁶ Further research showed that both turmeric extract (TE) and turmeric oil (TO) had potential anticancer activity. They also showed that the mutagenic effect of Benzopyrene BP, in the mouse bone marrow cells, was suppressed by Turmeric.^{7,8} Turmeric extract (TE) is bulky and difficult to take in the amounts recommended. Hence it was decided by the investigators, after reviewing animal toxicity and efficacy data, to use TO in capsule form so that long term administration is feasible and acceptable. SMF is a known precancerous condition for oral cancer and treatment modalities for reversing the changes have not been successful so far.⁹ Bhide

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and Jakhi reported symptomatic relief and clinical improvement in the opening of jaw with TE and TO in 30 cases of SMF in a pilot study.¹⁰ The interincisural opening increased in 16/21 cases treated with TO and in 8/10 cases treated with TE. The group also reported the protective effects of turmeric on micronuclei production in peripheral lymphocyte cultures and in buccal mucosal smears from human subjects with SMF. They further confirmed the reversal of cytogenetic damage by TO and turmeric oleoresin (TOR) as indicated by a reduction in the number of micronuclei in exfoliated buccal mucosal smears from patients of SMF from a mean of 10.2 ± 0.28 a (mean \pm SD) to a 3.9 ± 0.23 a (mean \pm SD). A therapeutic response to the symptoms of burning sensation or difficulty in opening the jaw was also reported.¹¹⁻¹³ Although lifelong use of turmeric for dietary cooking purposes and prolonged use as traditional medicine is shown to be safe, predominantly in India, and to a certain extent in other countries also, preclinical toxicity data was generated under the ICMR collaborating center, Sion LTMC, Mumbai, according to the ICMR guidelines. These data were submitted to the Drug Controller of India and the present study was approved by the DCI, ICMR and the Ethics Committee of Bhavan's SPARC.

Objectives : a) To determine the dose-related safety of TO in volunteers based on symptoms, signs and organ function tests. b) To evaluate the effects of TO on micronuclei in the human blood lymphocytes.

Design : Open labelled dose searching study of TO with an in-depth baseline and follow up investigation in ambulant volunteers for a period of 3 months.

MATERIAL AND METHODS

Formulation : Gelatine capsules containing 0.2ml of TO were prepared under aseptic conditions. The composition of TO was as follows :

- | | |
|------------------------------------|------|
| 1. Turmerone and arturmerone | 59% |
| 2. Zingiberene | 25% |
| 3. Cineole | 1% |
| 4. d - phellandrene | 1% |
| 5. d - sabinene | 0.6% |
| 6. Borneol | 0.5% |
| 7. α and β - allatone | |
| 8. Sesquiterpene alcohol | |

TO was diluted with maize starch. Accelerated stability studies were carried out at 2° to 8° Celsius, and 25° C and 37° C at room temperature and at 55% and 75% relative humidity using TLC markers observed under short uV at 254 nm.

Dose : Two doses were studied. Initially for 1st month all volunteers received a dose of 0.6ml of TO per day in three divided doses of one capsule of 0.2 ml each. After one month they received an increased dose of 1 ml of TO per day for two months in the form of two capsules in the morning and evening and one in the afternoon.

TO intake : This was supervised throughout the study

period except on Sundays when the volunteers were given the week-end doses.

Subject selection : Four clinically healthy males and five females participated in the study after giving an informed consent. None of them was married. They did not give any history of disease or surgery within the past six months. There was no history of drug allergy. There was no history of smoking, chewing tobacco in any form, alcoholism or other drug addiction.

Pretherapy investigations and Organ function tests : All volunteers underwent a complete medical history record and physical check up on a specially designed case record form for the phase I study. Their chest X-ray, electrocardiogram and routine urine examination were within normal limits before starting the study. They were negative for HIV and HBV infections. The following clinical chemistry was carried out : fasting blood sugar, haemoglobin, haematocrit, red cell count, white cell count, differential count, platelet count, bleeding time, clotting time, prothrombin time, blood urea nitrogen, serum creatinine, serum uric acid, serum albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total and direct bilirubin, total cholesterol, triglycerides, HDL and LDL cholesterol and serum potassium, sodium and chloride levels. Standard laboratory techniques were used with appropriate quality control. The biochemical investigations were within normal limits for the volunteers before starting the study. These were repeated at 4 weeks and at 12 weeks.

Lymphocyte culture : In view of the earlier work done at Bhavan's SPARC, blood lymphocyte cultures and micronuclei counts were examined as markers of DNA damage in all volunteers at initial examination and at 3 months as described in our previous study for micronuclei.¹⁰⁻¹¹

Early tolerance profile : All volunteers underwent complete clinical examination at 2 weeks, 4 weeks, 8 weeks and 12 weeks. Daily interviews were held to record any untoward events. Drug intake was supervised by the investigators in all volunteers throughout the study period except on Sunday. The higher dose of 1.0 ml was started after 1 month after observing that there were no side effects. This was continued for 2 months. During this period data of complete clinical examination and weight and blood pressure were recorded out at 4 weeks, and 12 weeks.

Acute tolerance profile : This consisted of continuous observation on the first day of TO intake for 8 hours' admission to the Clinical Pharmacology Unit. Baseline symptoms and signs were recorded.

Pulse, temperature and blood pressure were recorded at 0, 0.5, 1, 2, 4, 6, 8, 24 and 48 hours in the supine and standing position in 5 volunteers.

RESULTS

Demographic profile : All volunteers were between 20 and 33 years of age (Mean age 23.8 ± 1.2 yrs) . Their mean height, weight, body surface area and body mass index were

Table 1: Effect of turmeric oil on blood pressure in Phase I clinical trial (Systolic/Diastolic mm Hg)

	Time cuts											
	0 hr		0.5 hr		1 hr		2 hrs		4 hrs		6 hrs	
	S	U	S	U	S	U	S	U	S	U	S	U
PN	110/70	110/80	108/80	110/80	110/70	110/74	116/82	114/80	110/68	112/70	112/70	110/74
BS	110/74	110/80	110/80	110/80	110/70	114/80	114/68	110/70	114/70	114/70	110/70	110/80
MV	120/84	106/80	110/76	114/84	120/84	116/88	114/80	114/78	112/76	112/86	120/74	110/76
RK	110/70	104/68	110/74	110/78	110/78	110/72	110/78	110/78	110/68	112/70	110/70	106/70
AB	112/74	110/76	112/80	114/80	116/80	112/82	118/80	120/82	106/64	112/68	112/64	110/70
NS	114/64	116/72	110/68	106/70	108/66	110/70	94/58	94/60	108/64	104/64	120/60	110/62
PK	108/70	104/68	108/76	106/74	104/76	108/80	110/70	104/72	108/74	104/70	110/68	104/78
PR	110/68	110/66	108/60	104/60	102/56	104/60	110/56	104/58	112/56	102/54	124/60	114/68
DA	110/70	110/86	110/82	110/84	108/64	110/80	108/74	104/80	100/70	104/70	104/70	100/74

S : Supine; U : Upright

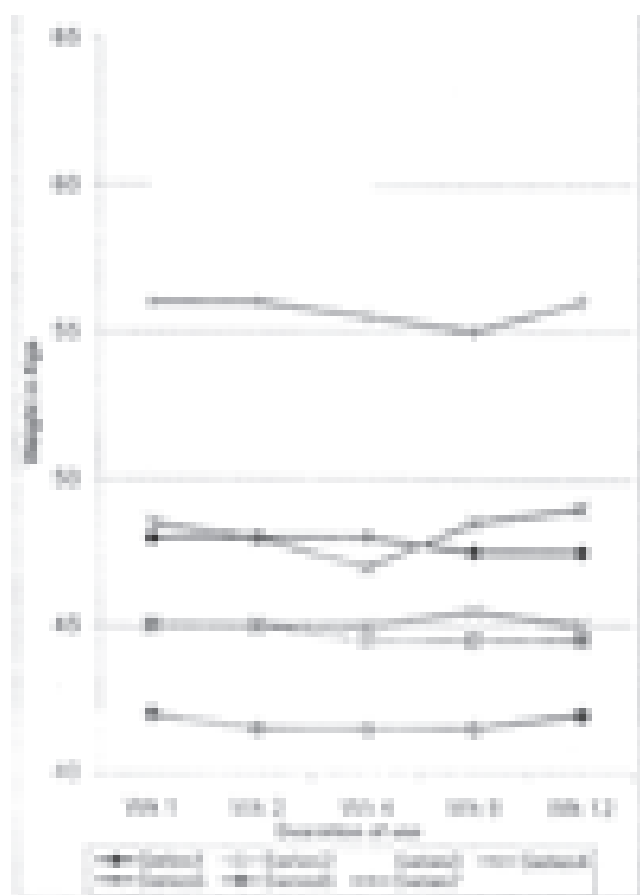


Fig. 1 : Weight of volunteers during phase I study of TO; no significant change noticed.

161.3 ± 3.0 cms, 50.4 ± 2.5kg, 1.51 ± 0.4 sq.m and 19.4 ± 0.8 Kg/m² respectively. None of them gave history of tuberculosis, heart disease or any major illness or surgery in the past. One volunteer No. 9 gave history of asthma in childhood but was not on any medication for the last 26 years.

Early Tolerance : The vital parameters like pulse and respiration were within normal limits during continuous observation on Day 1, and at 24 and 48 hours were within normal limits. The blood pressure in supine and standing or upright positions was also within normal limits as shown in Table 1.

Clinical Tolerability upto 3 Months : Two volunteers discontinued TO study after 1 week, one for allergic rash and the second for incidental fever which was later confirmed to be tuberculosis. The remaining seven volunteers completed the study without any major untoward event. They were all employed and continued with their normal duties. The following minor complaints were recorded during the study period of 3 months: a short episode of cough lasting for 1 or 2 days (N = 2), cold (N = 2), abdominal discomfort (N = 1), abdominal cramp (N = 1), headache (N = 1) and conjunctivitis for 3 days (N = 1).

Volunteer No. 1, female, developed fever, pain in abdomen, giddiness and cervical lymphadenopathy on 5th day of the TO intake. She discontinued TO on 7th day for this intercurrent incidental fever which was later confirmed to be tuberculous lymphadenopathy for which she underwent complete treatment subsequently. Volunteer No 9 developed itching and maculopapular rash on the trunk on 2nd day of treatment. This did not respond to lactocalamine and cetirizine (2 mg) dose. Lesions became worse on 7th day and TO was discontinued. Subsequently the rash healed gradually over 8 weeks.

Seven other volunteers remained well throughout the study period. Four of them expressed a distinct sense of well being and had improved appetite. Two female volunteers reported improved complexion of skin and one reported reduced dark circles around the eyes which was confirmed by the observers.

The menstrual pattern remained normal in all the three female volunteers.

Effect on Weight : There was no significance change in the weight of the volunteers at 1 or 2 days and at weekly follow ups (Fig 1).

Haematological Tolerability : There was no change in the haemoglobin, haematocrit, red cell count and total and differential white blood cell counts in any volunteer during TO treatment over 3 months. There was no effect of TO on bleeding time, clotting time and prothrombin time (Table 2).

Liver Functions: Serum levels of AST, ALT, ALP, albumin, direct and indirect bilirubin were normal initially and remained

Table 2 : Effect of turmeric oil on haemogram and coagulation profile in Phase I clinical trial (n = 7)

Tests	N. Ranges	Time Cuts		
		Basal	4 Weeks	12 Weeks
Haemoglobin (g %)	[12 - 18]	13.1 ± 2.7	13.4 ± 0.4	13.3 ± 0.4
Haematocrit (cc %)	[35 - 55]	50.0 ± 1.9	49.2 ± 1.4	49.8 ± 0.8
RBC (cmm)	[4.5 - 6.0]	4.09 ± 0.15	4.16 ± 0.14	3.96 ± 0.12
Total WBC (cmm)	[5000 - 10,000]	6182 ± 213	6742 ± 164	6557 ± 152
Clotting time	[3 - 11 minues]	4.71 ± 0.22	5.60 ± 0.37	4.18 ± 0.11
Bleeding time	[1 - 3 minutes]	1.90 ± 0.16	2.26 ± 0.20	2.26 ± 0.22
Prothrombin time	[control = 13 seconds]	15.2 ± 0.4	14.6 ± 0.5	12.8 ± 0.3

Differences as not statistically significant. Values are expressed as mean ± SE

Table 3 : Effect of turmeric oil on lipid profile in the volunteers (n = 7) of Phase I clinical trial

Tests	Normal Range	Time Cuts		
		Basal	4 Weeks	12 Weeks
Fasting sugar (mg %)	[65 - 95]	67.4 ± 1.9	65.8 ± 1.4	64.2 ± 1.8
Serum cholesterol (mg %)	[130 - 250]	156 ± 09	153 ± 09	186 ± 19
Serum triglycerides (mg %)	[40 - 170]	86.4 ± 8.80	83.2 ± 10.8	61.7 ± 7.2 [@]
Serum HDL-cholesterol (mg %)	[30 - 75]	58.6 ± 4.2	54.9 ± 2.9	55.2 ± 5.0
Serum LDL-cholesterol (mg %)	[upto 150]	80.2 ± 7.8	81.8 ± 7.2	124.1 ± 16.0 [@]

[@] : Paired 't' test : p < 0.05

In one volunteer TG values were 110 and 40 and LDL were 112 and 205 at 4 wks, and 12 wks respectively. On excluding this volunteer, mean TG values were 78 and 65 and LDL values were 76.7 and 110.6 at 4 and 12 wks respectively.

within normal limits during therapy (Fig 2).

Renal Functions : The blood urea nitrogen, serum creatinine and serum uric acid levels remained within normal limits throughout the treatment period of 3 months.

Carbohydrate and Lipid Profile : The fasting blood sugar remained normal in all subjects. Serum levels of cholesterol, triglycerides and LDL cholesterol were not altered in six out of seven volunteers who completed the study (Table 3). Three volunteers showed a nonsignificant decrease in triglyceride levels. One volunteer (No 5) showed normal serum triglycerides and LDL initially and at 4 weeks but elevated levels at 12 weeks. This volunteer was followed up for 1 month after discontinuation of TO and serum lipids showed return to normal.

Serum Electrolytes : Serum sodium and potassium levels of the volunteers remained within normal range throughout

Table 4 : Effect of turmeric oil on serum electrolytes in Phase I clinical trial (n = 7)

Tests	Duration of Treatment		
	Basal	4 weeks	12 weeks
Serum Na ⁺ (mEq/L) [1.5 - 7.0]	130.0 ± 0.8	132.0 ± 1.1	135.7 ± 3.0
Serum K ⁺ (mEq/L) [3.8 - 5.0]	4.3 ± 0.1	4.8 ± 0.2	4.3 ± 0.2
Serum Cl ⁻ (mEq/L) [99 - 110]	100.1 ± 1.0	101.8 ± 0.8	91.0 ± 1.4 [@]

[@] : Pair 't' test was used (p < 0.01); [] : Normal ranges; Values are expressed as mean ± S.E

Table 5 : Effect of turmeric oil on number of micronuclei in circulating lymphocytes in Phase I clinical trial (n = 7)

Volunteer no.	No. of Micronuclei/100 BNC		
	Basal	4 Weeks	12 Weeks
1	1.6	1.6	1.3
2	1.3	1.3	1.3
3	1.6	1.3	1.3
4	1.3	1.3	1.3
5	1.3	1.3	1.3
6	1.6	1.6	1.3
7	1.6	1.6	1.3
Mean ± S.E.	1.4 ± 0.06	1.4 ± 0.06	1.3 ± 0.0

Differences are not statistically significant. BNC: Binucleated cells.

the study. Serum chloride showed a minimal but statistically significant decrease in level (p < 0.05 Student's paired 't' test) at 12 weeks but not at 4 weeks (Table 4). Weakness, lethargy, myalgia, muscle cramps were not reported by any volunteer. On the other hand as already reported four volunteers reported a distinct sense of well being.

Micronuclei Counts : The lymphocyte cultures from all volunteers were examined for micronuclei counts before and after 12 weeks of TO treatment. The counts remained normal (<2%) in all the volunteers (Table 5).

Physical Examination : Except for the two volunteers who

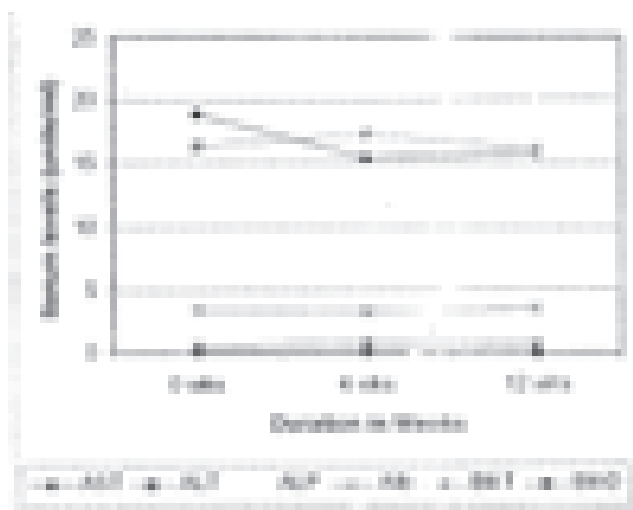


Fig. 2 : Hepatic tolerability of turmeric oil in phase I study; no significant changes in LFT.

discontinued treatment in the first week (one for allergic rash, one for intercurrent fever) none of the other volunteers developed any positive signs. All these volunteers maintained normal pulse, blood pressure and weight throughout the study period; no lymphadenopathy or hepatosplenomegaly was detected.

DISCUSSION

Oral cancer is quite common in India. Primary prevention by avoiding the use of tobacco in any form and good oral hygiene are ideal. However already several thousands of persons are affected by oral submucous fibrosis (SMF), a precancerous condition. Earlier attempts to treat or reverse SMF have not been fruitful.⁹

Turmeric extract and turmeric oil have demonstrated oncopreventive activity 'in vitro' and 'in vivo' in animal experiments by Bhide *et al*, and other workers.⁶⁻⁸ Initial studies conducted at Bhavan's SPARC in collaboration with the Nair Dental Hospital have shown relief of burning sensation and partial reversal of opening of the mouth as measured by the calipers and a reduction of micronuclei in the buccal smears when TO was administered for 3 months. The local symptoms of burning sensation and pain were reduced.¹⁰⁻¹³ Although the safety of crude turmeric powder use is known from our Ayurvedic texts, traditional use and its use as a daily household spice it was necessary to conduct Phase I and clinical tolerability studies in volunteers before studying TO for chemoprevention of cancer in SMF.

The present study has shown that TO in doses of 0.6ml and 1.0 ml is safe in clinical use upto 3 months of continuous daily intake. There were no acute side effects. In one case allergic skin reaction to the TO preparation was observed. There was no change in the menstrual pattern of female volunteers. Only one case had reversible hypertriglyceridemia. Other cases exhibited reduced lipid levels in corroboration with an earlier experimental study.¹⁴ All organ function tests were within normal limits throughout the study. Serum chlorides were slightly reduced in the volunteers. However none of the seven volunteers who completed the study complained any general side effects. On the contrary four reported a feeling of well being and two females reported improved skin complexion. This is consistent with the therapeutic properties and uses of turmeric described in Ayurveda.¹⁻³

The mechanism of action of TO in oncoprevention can be speculated to be through its antioxidant action and protection against DNA damage. It has been shown to favourably alter the serum glutathione and superoxide dismutase activity and reduced lipid peroxidation by studies in patients of SMF and in controlled animal experiments. Hepatoprotective action has also been shown in carbontetrachloride induced hepatic damage in rats.^{15,16} Several recent studies have confirmed the antioxidant and DNA protective effect of *Curcuma longa*.¹⁷⁻²⁰

It has also been shown to inhibit the proliferation of vascular smooth muscle cells in tissue culture and may prevent

neoangionesis in carcinogenesis. Curcumin promotes apoptosis.²⁰⁻²² Kuttan *et al* have reported remarkable symptomatic relief with topical application of ethanol extract of turmeric and curcumin ointment in 70% of the 62 cases with external cancer.²³

An adverse effect on lipids was noticed in only one case in this study. Prakasunand *et al* have reported on the biochemical safety of turmeric, 3gms/day, in 54 cases of peptic ulcer.²⁴

The composition of essential oil is likely to be variable as reported by Martins *et al*.²⁵ Thus standardization for TO contents will be of crucial importance in dose determination.

The healing effect of turmeric has been described in Ayurveda and other traditional systems. This has also been confirmed by several experimental studies and probably accounts for not only arrest of carcinogenesis but reversal of the SMF seen in the pilot study. The findings need confirmation in a larger Phase II trials. The anti-inflammatory, antimicrobial, wound healing properties, and other benefits of *C. longa* are well known. Most studies have been conducted on curcumin. The oncoprotective effect of TO requires further evaluation in closely monitored human clinical trials..There have been no side effects or complications of changes in chloride levels reported so far.

CONCLUSIONS

From earlier experimental and clinical data and the results of extended clinical Phase I trial of turmeric oil for 3 months it can be concluded that Turmeric extract oleoresin or turmeric oil has potential as a chemoprotective agent, particularly in patients of oral submucous fibrosis, which is otherwise irreversible and potentially precancerous.

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