Turk J Pharm Sci 2019;16(1):27-31
DOI: 10.4274/tjps.77598

ORIGINAL ARTICLE

Preclinical Evaluation of the Haematinic Activity of an Oral Indiffusible Mixture of *Tamarindus indica* L. Leaf Extract

Tamarindus indica L. Yaprak Ekstresinden Hazırlanan Oral Dağılmayan Karışımın Hematinik Etkisinin Preklinik Değerlendirmesi

Sathiya RAMU*, Shwetha Krishna MURTHY, Sukanya KRISHNA, Abhishek Lakkasandra SOMASHEKARAIAH, Vani B. NANDIHALLI, Kanekal Mohammed MUZAMMIL

M. S. Ramaiah University of Applied Sciences, Faculty of Pharmacy, Karnataka, India

ABSTRACT

Objectives: *Tamarindus indica* L. is known to be a multipurpose traditional plant in India. It is used to treat some bacterial infections, parasitic infestations, constipation, and inflammation. It is also used as a blood tonic and for wound healing. This study was designed to substantiate the traditional claim of haematinic activity for *T. indica*.

Materials and Methods: *T. indica* leaf extract was formulated into an oral indiffusible mixture (TIM) and evaluated for its haematinic activity in phenylhydrazine (single dose of 10 mg/kg *per oral* for 8 days) induced anaemia. Wistar rats were grouped into six (n=6). Groups I and II served as normal control and disease control groups, respectively. Group III received the standard drug (haematinic suspension 2 mL/kg). Groups IV, V, and VI received the formulated oral indiffusible mixture of *T. indica* at a dose of 100, 200, and 400 mg/kg, respectively.

Results: The TIM was formulated and pharmaceutically optimized. It produced significant increases in red blood cells, hemoglobin, and packed cell volume and a decrease in mean corpuscular volume.

Conclusion: The results showed that the treatment with TIM reversed phenylhydrazine induced anemia. However, the short duration of the present study is regarded as a limitation, and therefore a longer duration is required for obtaining better responses.

Key words: *Tamarindus indica*, haematinic activity, phenylhydrazine

ÖZ

Amaç: *Tamarindus indica* L. Hindistan'da çok amaçlı kullanılan geleneksel bir bitki olarak bilinmektedir. Bazı bakteriyel ve parazit enfeksiyonlarının tedavisinde, kabızlık ve inflamasyonun tedavisinde kullanılmaktadır. Kan toniği ve yara iyileştirici amaçla da kullanımı kayıtlıdır. Bu çalışma, *T. indica*'nın geleneksel kullanımının doğruluğunun araştırılması amacıyla yapılmıştır.

Gereç ve Yöntemler: *T. indica* yaprağından hazırlanan ekstre oral dağılmayan karışım (TIM) halinde formüle edilmiş ve fenilhidrazin-nedenli (8 gün boyunca 10 mg/kg *tek doz, oral*) anemi modeli üzerindeki hematinik etkisi değerlendirilmiştir. Wistar sıçanları altı gruba ayrılmıştır (n=6). Grup I ve II sırasıyla normal kontrol ve hastalık kontrol grupları olarak ayrılmıştır. Grup III'e standart ilaç (hematinik süspansiyon 2 mL/kg) verilmiştir. Grup IV, V ve VI'ya, sırasıyla 100, 200 ve 400 mg/kg'lık dozlarda oral dağılmayan karışım şeklinde formüle edilmiş olan *T. indica* ekstresi uygulanmıştır.

Bulgular: TIM formüle edilmiş ve farmasötik olarak optimize edilmiştir. Bu karışımın kırmızı kan hücreleri, hemoglobin ve paketlenmiş hücre hacminde önemli artış ve ortalama kan hacminde azalmaya neden olduğu bulunmuştur.

Sonuç: Bu sonuçlar, *T. indica* yaprak ekstresinden hazırlanan TIM'nin, fenilhidrazin-nedenli aneminin etkilerini tersine çevirdiğini göstermiştir. Bununla birlikte, bu çalışmanın kısa süreli olması bir sınırlama olarak kabul edilmiştir, bu nedenle daha iyi yanıtlar elde edebilmek için daha uzun bir süreye ihtiyaç duyulmaktadır.

Anahtar kelimeler: *Tamarindus indica*, hematinik etki, fenilhidrazin

*Correspondence: **E-mail:** sathya.pharma@gmail.com, **Phone:** 9035919823 **ORCID-ID:** orcid.org/0000-0003-3474-7033
Received: 13.09.2017, **Accepted:** 07.12.2017

INTRODUCTION

All through history, irrespective of culture, plants have been a dependable source of medicine.[1,2] Plants and their derived products are considered to be the main source for food and medicines. Plant derived medicines, popularly known as "herbal drugs" or "phytomedicines", are well known and accepted as the most common form of alternative medicine. Almost 70-90% of the world's rural population still depends on herbal remedies for health care.[3] Plants produce a good deal of secondary metabolites that have benefited mankind in various ways, including treatment of diseases.[4] They are mostly used in Ayurveda, Unani, Siddha, homeopathy, allopathy, and other alternative medicinal practices.[5]

Anaemia is defined as a reduction in haemoglobin level and oxygen carrying capacity below the normal range and is the most common disorder of the blood. It is characterised by a decrease in haemoglobin level to less than 13 g/dL in males or 12 g/dL in females.[6] In anaemia the rate of production of mature red blood cells entering the blood from the red bone marrow does not keep pace with the rate of haemolysis.[7] Iron is the main constituent of haemoglobin, which is accountable for transporting oxygen, and of myoglobin in muscles and is part of many enzymes concerned with cellular processes, respiration, and cell division.[8] Low haemoglobin (Hb) levels result in a corresponding decrease in the oxygen carrying capacity of blood[7] and other parameters such as total red blood cell (RBC) count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and MCH concentration (MCHC).[7,9]

Tamarindus indica is the third largest family of flowering plants, with a total of 727 genera and 19,327 species.[10] *T. indica* is known to have mild laxative, preservative, and anti-measles effects due to the presence of tartaric acid and malic acid.[11] Polysaccharide obtained from *T. indica* pulp showed significant antipyretic activity against bacterial pyrogen.[12] Aqueous fruit extract of *T. indica* was shown to possess both central and peripherally acting analgesic activity.[13] Traditionally, *T. indica* leaves are used as a blood tonic[14] and for their wound healing, antimalarial, aphrodisiac, antihistaminic, antitussive, anti-inflammatory, antidiabetic, hepatoprotective, and antimeasles properties.[11] The bark and stem possess anti-asthmatic, antitussive, anti-inflammatory, astringent, hepato-protective, anthelmintic, and abdominal pain relieving effects.[11] The antioxidant property (flavonoids) of leaves of *T. indica* and also the existence of iron content in the leaves of *T. indica* have been previously reported.[15-17] Taking this into consideration, the present study was undertaken to substantiate the traditional claim of haematinic activity for leaves of *T. indica* in phenylhydrazine (PHZ) induced anaemia in Wistar rats.

MATERIALS AND METHODS

Plant material and preparation of T. indica leaf extract

Fresh leaves of *T. indica* were collected in the field of the KMF Society Hostel farm, Bangalore, Karnataka. The plant material was identified and authenticated by Dr. S. N. Yoganarasimhan, plant taxonomist. The taxonomic identification was carried out following Flora of the Presidency of Madras (2005), Flora of Hassan District (1976), and Flora of Bombay (1967). The voucher specimen was deposited at the herbarium of the Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore. The plant material was shade dried, coarsely powdered, and stored in an airtight container. These shade dried leaves were extracted with 95% v/v ethanol in a Soxhlet apparatus. The alcohol extract was filtered, the solvent was evaporated, and accurate weight of the extract was recorded. The colour and consistency of the extract were noted.

Phytochemical screening

Preliminary phytochemical screening of *T. indica* extract involved qualitative determination of the following substances: alkaloids, carbohydrates, glycosides, phytosterols, phenolic compounds, tannins, saponins, terpenes, and flavonoids. It was carried out in accordance with procedures described by Kokate.[18]

Formulation of oral indiffusible mixture

The ethanolic extract of *T. indica* leaf was formulated into an oral indiffusible mixture by hydrating overnight an accurately weighed quantity of ethanolic extract of *T. indica* and cross povidone (1%) solution. Sodium CMC (2%) was taken in separate beaker and kept for overnight hydration. These mucilages were put into a mortar along with glycerine (10%) and triturated to obtain a uniform mixture. Calcium chloride (0.8%) was added dropwise to the above mixture and it was triturated continuously until a uniform dispersion of extract was obtained. The prepared formulation was transferred to a measuring cylinder and the volume was adjusted. Three formulations were prepared as per the dose required for the pharmacological studies[19,20] (Table 1). Formulation codes are given as follows: TIM1 - 100 mg/kg (15.8 mg/mL), TIM2 - 200 mg/kg (31.6 mg/mL), TIM3 - 400 mg/kg (54 mg/mL).

The oral indiffusible mixture of *T. indica* leaf ethanolic extract was evaluated for pH using a digital pH meter, viscosity using a Brookfield viscometer,[21] redispersibility,[19] flow rate (F) using

Table 1. Formulation of TIM

Sl. no.	Ingredients	TIM1	TIM2	TIM3
1	Ethanolic extract (g)	8	18	27
2	Sodium CMC (2%) (g)	10	10	10
3	Cross povidone (1%) (g)	5	5	5
4	Glycerine (10%) (mL)	50	50	50
5	Calcium chloride (0.8%) (g)	4	4	4
6	Methyl paraben (g)	0.5	0.5	0.5
7	Propyl paraben (g)	0.5	0.5	0.5
8	Raspberry flavour (mL)	5	5	5
9	Amaranth solution (g)	0.02	0.02	0.02
10	Purified water (q.s.)	Make up to 500 mL		

TIM: *T. indica* mixture, CMC: Carboxymethyl cellulose

a 10 mL pipette, particle size measurement using an Olympus microscope,[22] and sedimentation volume using a 100 mL measuring cylinder.[23,24]

Experimental animals

Wistar rats of 8-12 weeks old, weighing between 140 and 230 g of either sex were used for the study. The animals were bred, reared, and housed in the animal house of the Department of Pharmacology, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences. The animal house was well maintained under standard hygienic conditions, at a temperature of 22±2°C and room humidity of 60±10%, with 12-h day and night cycle, and with food and water *ad libitum*. Paddy husk was provided as bedding material and cleaning was done on alternate days. The animals were housed in groups of 3 per cage. The pharmacological study was approved by the Institutional Animal Ethics Committee of the Faculty of Pharmacy (IAEC certificate no. Ref. No. MSRCP/SP-51/2014).

Acute toxicity study

The oral indiffusible mixture of ethanolic extract of *T. indica* leaf was screened for its toxicity following the OECD guidelines 423. A limit test was carried out with a dose of 2000 mg/kg in 3 female Wistar rats.[25]

Experimental design

Anaemia was induced by oral administration of PHZ at a dose of 10 mg/kg per day for 8 days. The animals were divided into six groups. Each group consisted of six animals of either sex.

Groups I and II served as normal control and disease control groups, respectively. Group III received the standard drug (haematinic suspension 2 mL/kg). Groups IV, V, and VI received formulated oral indiffusible mixture of *T. indica* at a dose of 100, 200, and 400 mg/kg, respectively.

The animals were treated once daily for 14 days with different doses of the oral indiffusible mixture. After day 14 of treatment, blood was collected from the retro-orbital plexus under light ether anaesthesia from overnight fasted experimental animals. Physical parameters (body weight and food and water intake) were evaluated during treatment of the animals. Haematological parameters including RBC and Hb were estimated using automatic analysers. PCV was evaluated by centrifugation. MCV, MCH, and MCHC were calculated using the standard formulae according to Ghai.[26]

Statistical analysis

The results of haematinic activity of the oral indiffusible mixture of *T. indica* leaf extract were subjected to statistical analysis. The data were expressed as mean ± standard error mean. Significant differences between groups were determined using one-way ANOVA followed by Tukey's multiple comparison; $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The phytochemical screening of *T. indica* revealed the presence of alkaloids, flavonoids, saponins, phenols, oils and fatty acids, carbohydrates, and tannins.

Evaluation of the oral indiffusible mixtures

The oral indiffusible mixtures were evaluated for pH, redispersibility, flow rate, particle size, viscosity, and sedimentation volume. The results of these parameters are reported in Tables 2 and 3. The pH of these formulations was in the range of 4.5-4.8, which is slightly acidic. In sedimentation TIM3 showed greater sedimentation volume when compared to the other two formulations. Slightly higher viscosity was observed in the higher dose formulation. The flow rate of the mixtures was in the range of 0.10-0.14. Particle size was determined using a microscope and was between 215 and 230 µm (Table 2). After the complete sedimentation of the suspension, formulations were redispersed. In that, the TIM1 formulation took fewer cycles to redisperse (Table 3).

Acute toxicity

A limit test was carried out following OECD guidelines 423. The results are reported in Table 4. All the animals were free of intoxication signs and there were no signs of mortality in the acute toxicity study (Table 4).

Table 2. Evaluation parameters of TIM1, TIM2, and TIM3 formulations

Sl. no.	Parameters	TIM1	TIM2	TIM3
1	pH	4.5±0.2	4.8±0.1	4.5±0.2
2	Redispersibility	3 times	4 times	6 times
3	Flow rate (mL/s)	0.1388±0.002	0.1250±0.005	0.1041±0.003
4	Particle size (µm)	220±25	230±29	215±35
5	Viscosity (cp)	9.0±0.23	12.6±0.43	13.5±0.31

TIM: *T. indica* mixture

Table 3. Sedimentation volume of different formulations

Formulation	$F=V_u/V_o$							
	1 h	2 h	6 h	12 h	1st day	2nd day	3rd day	4th day
TIM1	1	0.96	0.91	0.88	0.85	0.81	0.78	0.74
TIM2	1	0.96	0.90	0.87	0.83	0.78	0.75	0.70
TIM3	1	0.95	0.90	0.85	0.81	0.77	0.75	0.70

TIM: *T. indica* mixture

Haematinic activity

The haematological parameters of the experimental animals after treatment with oral indiffusible mixtures of *T. indica* leaf extract are presented in Table 5. PHZ treated animals showed reductions in the levels of RBC and Hb, while MCV and MCHC increased significantly, resulting in macrocytic anaemia. There was also a slight increase in MCH, which supports the induction of macrocytic anaemia by PHZ. Fourteen day treatment of anaemic rats (groups IV, V, and VI) with oral indiffusible mixture

Table 4. Acute toxicity results

Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000 mg/kg	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–

1. Alertness, 2. Aggressiveness, 3. Pile erection, 4. Grooming, 5. Gripping, 6. Touch response, 7. Decreased motor activity, 8. Tremors, 9. Convulsions, 10. Muscle spasm, 11. Catatonia, 12. Muscle relaxant, 13. Hypnosis, 14. Analgesia, 15. Lacrimation, 16. Exophthalmos, 17. Diarrhoea, 18. Writhing, 19. Respiration, 20. Mortality

Table 5. Haematinic activity of oral indiffusible mixture of *T. indica* leaf extract

Parameters	Normal control	Disease control	Standard	TIM1	TIM2	TIM3
RBC (millioncells/mm³)	5.68±0.07	5.06±0.17[a]	5.75±0.12*	5.46±0.14	5.68±0.19*	5.48±0.11
Hb (g/dL)	17.32±0.23	15.31±0.5[a]	17.33±0.33*	16.50±0.42	17.15±0.57*	16.58±0.33
PCV	58.68±1.72	51.69±1.70	58.85±2.78	52.40±3.02	52.96±3.51	57.90±1.30
MCV	96.30±1.99	102.1±0.78[a]	102.1±2.08	95.49±3.57	92.67±3.24*	105.6±0.41
MCH	30.19±0.04	30.20±0.09	30.15±0.07	30.18±0.06	30.18±0.07	30.24±0.06
MCHC	35.08±0.85	29.61±0.17[a]	29.66±0.87	32.72±1.24	32.72±1.24	28.64±0.12

RBC: Red blood cell, Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Values are expressed as mean ± standard error mean; n=6; [a]p<0.001 in comparison with normal control; *p<0.05 in comparison with disease control

of *T. indica* leaf extract reversed the effect of PHZ induced anaemia. The treatment resulted in a significant increase in the level of RBC and Hb ($p<0.05$) and a significant decrease in MCV ($p<0.05$).

Table 5 represent the changes in RBC, Hb, PCV, MCV, MCH, and MCHC in each group after 14 days of treatment. Administration of PHZ resulted in megaloblastic anaemia characterised by decreases in RBC, Hb, and PCV. Treatment for 14 days with the oral indiffusible mixture of *T. indica* leaf extract reversed the effects of PHZ induced anaemia. There were also increases in MCV and MCH due to PHZ, which indicated macrocytic anaemia. The recovery time for the haematological parameters was low for the lowest dose but there was progressive recovery in RBC, HB, and PCV after 14 days.

All three formulations showed increases in RBC, Hb, and PCV and a decrease in MCV. There was a statistically significant improvement in the level of RBC and Hb ($p<0.05$) after treatment with TIM2 (200 mg/kg). The improvement seen after treatment with TIM2 was comparable with that of the standard drug. There was no further increase in the levels of RBC or Hb with TIM3 (400 mg/kg). This shows that the response to treatment with the oral indiffusible mixture of *T. indica* leaf extract was dose related. TIM1 caused increases in RBC, Hb, and PCV to submaximal levels when compared to TIM2. There were no large changes in MCH in the groups including the standard, whereas MCV significantly decreased ($p<0.05$) after treatment with TIM2. However, the short duration of the present study can be considered a limitation; therefore, a longer duration is required for obtaining better responses.

CONCLUSIONS

It is postulated that the presence of flavonoids, phenols, and iron in herbal extracts is responsible for haematinic activity. The oral indiffusible mixture of *T. indica* leaf extract significantly increased the haemoglobin and RBC count in anaemic rats, indicating haematinic activity at a dose of 200 mg/kg. Thus, the oral indiffusible mixture of *T. indica* L. leaf extract was proven to possess haematinic activity. Further studies are needed to elucidate the mechanism (s) involved in the haematinic activity.

ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, for providing the required facilities and support.

Conflict of Interest: No conflict of interest was declared by the authors.

REFERENCES

1. Stockwell S. Nature's Pharmacy. London: Century Hutchinson Ltd; 1988.
2. Thomson. Medicines from the Earth. Maidenhead, UK: McGraw-Hill Book Co; 1978.
3. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem. 2004;11:1451-1460.
4. Souza-Fagundes EM, Queiroz AB, Martins Filho OA, Gazzinelli G, Corrêa-Oliveira R, Alves TM, Zani CL. Screening and fractionation of plant extracts with antiproliferative activity on human peripheral blood mononuclear Cells. Mem Inst Oswaldo Cruz. 2002;97:1207-1212.
5. Chaudhuri AB. Endangered Medicinal plants, New Delhi: Daya Publishing House; 2001.
6. Ogbe RJ, Aduga GI, Abu AH. Antianemic potentials of some plants extracts on phenyl hydrazine induced anemia in rabbits. J Medicinal Plants Res. 2010;4:680-684.
7. Waugh A, Grant A. Ross and Wilson Anatomy and Physiology in health and illness, UK: Elsevier Churchill Livingstone; 2006.
8. Benoist B, McLean E, Cogswell M, Egli I, Wojdyla D, Worldwide prevalence of anaemia, WHO Global Database on Anaemia World Health Organization, Geneva; 2008
9. Tortora GJ, Derrickson BH. Principles of Anatomy and Physiology, NJ: John Wiley and Sons; 2009.
10. Samina KK, Shaikh W, Sofia S, Kazi TK, Amina KK, Usmanghani K, Sheerazi TH, Chemical constituents of *T. indica* L in Sindh. Pak J Bot. 2008;40:2553.

11. Havinga RM, Hartl A, Putscher J, Prehsler S, Buchmann C, Vogl CR. *Tamarindus indica* L. (Fabaceae): patterns of use in traditional African medicine. J Ethnopharmacol. 2010;127:573-588.
12. Izquierdo T, Gracia - Tamayo F, Soto C, Castrillon LE. A *Tamarindus indica*. Linn pulp polysaccharide inhibits fever *in-vivo* and IL-1β release by murine peritoneal exudates cell. Pharmaceu Bio. 2007;45:22-30.
13. Khalid S, Shaik Mossadeq WM, Israf DA, Hashim P, Rejab S, Shaberi AM, Mohamad AS, Zakaria ZA, Sulaiman MR. *In Vivo* Analgesic effect of aqueous extract of *Tamarindus indica* L. Fruits. Med Princ Pract. 2010;19:255-259.
14. Doughari JH. Antimicrobial activity of *Tarindus indica* Linn. Trop J Pharmaceu Res. 2006;5:597-603.
15. Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G, Jain AP. *Tamarindus indica*: extent of explored potential. Pharmacog Rev. 2011;5:73-81.
16. De Caluwé E, Halamova K, Van Damme P. *Tamarindus indic*a L a review of traditional uses, Phytochemistry and Pharmacology. Afrika Focus. 2010;23:53-83.
17. Khairunnuur FA Jr, Zulkhairi A, Azrina A, Moklas MM, Khairullizam S, Zamree MS, Shahidan MA. Nutritional composition, *in vitro* antioxidant activity and *Artemia salina* L. lethality of pulp and seed of *Tamarindus indica* L. extracts. Malays J Nutr. 2009;15:65-75.
18. Kokate CK. Practical Pharmacognosy (4th ed). New Delhi; Vallabh prakashan; 1999.
19. Chukka S, Puligilla S, Yamsani MR. New formulation and evaluation of domperidone suspension. World J Pharmacy and Pharma Sci. 2014;3:1867-1884.
20. Dhanapal CK, Manavalan R, Chandar N, Chenthilnathan F. Formulation development of pediatric rifampicin oral suspension. Der Pharmacia Lettre. 2012;4:845-853.
21. Weiland RH, Dingman JC, Cronin DB, Browning GJ. Density and Viscosity of Some Partially Carbonated Aqueous Alkanolamine Solutions and Their Blends. J Chem Eng Data. 1998;43:378-382.
22. Gaikar NV, Sandhya P, Chaudhari CA. Evaluation of *Curculigo orchioides* Mucilage as Suspending Agent. Int J Pharm Tech Res. 2011;3:831-835.
23. Banker SG, Rhodes CT. Modern Pharmaceutics. New York, Marcel Dekker; 1998.
24. Patel NK, Kenon L, Levinson RS. The Theory and Practice of Industrial Pharmacy. (3rd) Indian Edn. Mumbai; Vargheese Publishing House;1986.
25. Ramachander T, Rajkumar D, Sravanprasad M, Goli V, Dhanalakshmi CH, Arjun. Antidiabetic activity of aqueous methanolic extracts of leaf of *Tamarindus indica*. Int J Pharm Phy Res. 2012;4:5-7.
26. Ghai CL. A Text book of Practical Physiology (6th ed). New Delhi; Jaypee Brothers; 2005.