Preclinical evaluation of haematinic activity of oral indiffusible mixture of *Tamarindus indica* L. leaf extract

ABSTRACT

Tamarindus indica L. *(T. indica)* is known to be a multipurpose traditional plant in India. It is used against some bacterial infections, parasitic infestations, constipation and inflammation. It is also used as a blood tonic and used for wound healing. This study was designed to substantiate the traditional claim of haematinic activity of *T. indica. T. indica* leaf extract was formulated into an oral indiffusible mixture (TIM) and evaluated for its haematinic activity in phenylhydrazine (Single dose of 10mg/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 respectively. Group III received standard drug (Haematinic suspension 2ml/kg). Groups IV, V, VI received formulated oral indiffusible mixture of *T. indica* at the dose of 100, 200 and 400 mg/ kg respectively. TIM was formulated and pharmaceutically optimized. TIM produced significant increase in RBC, Hb and PCV while decrease in MCV. These results conclude that the oral indiffusible mixture of *T. indica* leaf extract taken for investigation was found to reverse phenylhydrazine induced anaemia. However, short duration of present study is proposed to be a limitation, thereby longer duration is required for obtaining better responses.

Key words*: Tamarindus indica,* Haematinic activity, Phenylhydrazine.

INTRODUCTION

All through history, irrespective of culture, plants have been a dependable source of medicine (Stockwell, 1988; Thomson, 1978). Plants and their derived products are considered to be the main source for food and medicines. Plant derived medicines popularly known as “Herbal drug” or “phytomedicine” is 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 (Lai and Roy, 2004). Plants produce a good deal of secondary metabolites which have benefited mankind in various ways, including treatment of diseases (Elaine *et al*., 2002). They are mostly used in Ayurveda, Unani, Siddha, Homeopathy, Allopathy and other alternate medicinal practices (Chaudhuri, 2001).

Anaemia is defined as the reduction of haemoglobin level and oxygen carrying capacity below the normal range and is the most common disorder of the blood. It is characterised by the decrease of the haemoglobin level less than 13 g/dl in male or 12 g/dl in female (Ogbe *et al*., 2010). 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 (Waugh and Grant, 2006). Iron is the main constituent of the haemoglobin which is accountable for transporting oxygen, myoglobin in muscles and part of many enzymes which are concerned in cellular processes, respiration and cell division (Benoist *et al.,* 2008). The low Hb levels result in a corresponding decrease in the oxygen carrying capacity of blood (Waugh and Grant, 2006) and other parameters such as total no. of Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Mean corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean corpuscular Haemoglobin Concentration (MCHC) (Waugh and Grant, 2006; Tortora and Derrickson, 2009).

T. indica is the third largest family of flowering plants with a total of 727 genera and 19,327 species (Samina *et al*., 2008). *T. indica* is known to be mild laxative, preservative and anti-measles due to the presence of tartaric acid and malic acid (Havinga *et al.,* 2010). Polysaccharide obtained from *T. indica* pulp showed significant antipyretic activity against bacterial pyrogen (Izquierdo *et al*., 2007). Aqueous fruit extract of *T. indica* was shown to possess both central and peripherally acting analgesic activity (Khalid *et al.,* 2010). Traditionally, *T. indica* leaves are used as blood

tonic (Doughari, 2006), wound healer, antimalarial, aphrodisiac, antihistaminic, antitussive, anti-inflammatory, antidiabetic, hepatoprotective and anti-measles (Havinga *et al.,* 2010). Bark and stem possess anti asthmatic, antitussive, anti-inflammatory, astringent, hepato-protective, anthelmintic and abdominal pain reliever (Fresh bark) (Havinga *et al.,* 2010). The antioxidant property (flavonoids) of leaves of *T. Indica* and also the existence of iron content in the leaves of *T. indica* have been already reported (Bhadoriya *et al*., 2011; Agricultural Research Service, 2015; De Caluwé *et al*., 2010; Khairunnuur *et al*., 2009). Taking this into consideration, the present study is undertaken to substantiate the traditional claim of haematinic activity of leaves of *T. indica* in phenylhydrazine induced anaemia in Wistar rats.

MATERIALS AND METHODS

i) Plant material and preparation of *T. indica* leaf extract

The fresh leaves of *T. indica* were collected in the field of KMF Society Hostel farm, Banglore, 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 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. This shade dried leaves were extracted with 95% v/v ethanol in soxhlet apparatus. The alcohol extract was filtered, the solvent was evaporated and accurate weight of the extract was taken. The colour and consistency of the extract were noted down.

ii) 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. Procedures were carried out in accordance with procedures described by Kokate, 1999.

iii) 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 taken into a mortar along with glycerine (10%) and triturated to obtain uniform mixture. Calcium chloride (0.8%) was added dropwise to the above mixture and triturated continuously until 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. Formulation codes are given as follows, TIM1-100mg/kg (15.8mg/ml), TIM2-200mg/kg (31.6mg/ml), TIM3-400mg/kg (54mg/ml) (Chukka *et al.,* 2014; Dhanapal *et al.,* 2012).

Oral indiffusible mixture of *T. indica* leaf ethanolic extract was evaluated for pH using digital pH meter, viscosity using Brookfield viscometer (Weiland et al., 1998), Redispersibility (Chukka *et al.,* 2014), Flow rate (F) using 10ml pipette, Particle size measurement using Olympus Microscope (Gaikar NV et al., 2011), Sedimentation volume using 100ml measuring cylinder (Banker et al., 1998; Patel et al., 1986).

iv) Experimental animals

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

v) Acute toxicity study

The oral indiffusible mixture of ethanolic extract of *T. indica* leaf was screened for its toxicity following the OECD guidelines 423. Limit test was carried out with the dose of 2000 mg/kg in 3 female Wistar rats (Ramachander *et al., 2012*).

vi) Experimental Design

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

Wistar rats were grouped into six (n=6). Groups I and II served as normal control and disease control, respectively. Group III received standard drug (Haematinic suspension 2ml/kg). Groups IV, V, VI received formulated oral indiffusible mixture of *T. indica* at the dose of 100, 200 and 400 mg/ kg, respectively

The animals were treated once daily for 14 days with the different doses of oral indiffusible mixture. After 14th day of treatment, blood was collected from retro orbital plexus under light ether anaesthesia of overnight fasted experimental animals. Physical parameters (body weight, food and water intake were evaluated during treatment of animals. Haematological parameters including RBC and Hb were estimated using automatic analysers. PCV was evaluated by centrifugation method. MCV, MCH, MCHC were calculated using the standard formulae according to Ghai (2005).

vii) Statistical Analysis

The results of Haematinic activity of oral indiffusible mixture of *T. indica* leaf extract was subjected to statistical analysis. Data were expressed as mean ± SEM. Significant difference between groups were determined using One-Way ANOVA followed by Tukey's multiple comparison, $P < 0.05$ was considered as 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 oral indiffusible mixture

Oral indiffusible mixtures were evaluated for pH, redispersibility, flow rate, particle size, viscosity and sedimentation volume. The results of these parameters are reported in Table 2 and Table 3. The pH of these formulations were found to be in the range of

4.5-4.8, which is slightly acidic. In sedimentation the TIM3 shows more sedimentation volume when compare to the other two formulations. The slight higher viscosity was observed in the higher dose formulation. After the complete sedimentation of the suspension, formulations were redispersed. In that the TIM1 formulation took less cycles to redisperse. The flow rate of the mixtures were found to be in the range of 0.10 - 0.14. The particle size was determined using microscope, the range of particle lies in between 215-230µm.

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 also there were no signs of mortality in the acute toxicity study.

Haematinic activity

The haematological parameters of experimental animals after treatment with oral indiffusible mixture of *T. indica* leaf extract are presented in Table 6. PHZ treated animals showed reduction in the levels of RBC and Hb while the MCV and MCHC increased significantly resulting in macrocytic anaemia. There was also slight increase in MCH which supports the induction of macrocytic anaemia by PHZ. 14 days treatment of anaemic rats (Groups 4, 5 and 6) with oral indiffusible mixture of *T. indica* leaf extract reversed the effect of PHZ induced anaemia. The treatment resulted into a significant increase in the level of RBC, Hb ($p < 0.05$) and significant decrease in MCV ($p < 0.05$).

Figures 1 to 6 presents the changes in RBC, Hb, PCV, MCV, MCH and MCHC per group after 14 days of treatment. Administration of PHZ resulted in megaloblastic anaemia characterised by decrease of RBC, Hb and PCV. Treatment 14 days with oral indiffusible mixture of *T. indica* leaf extract reversed the effects of PHZ induced anaemia. There was also increase in the 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 a progressive recovery of RBC, HB and PCV after 14 days.

All the three formulations showed increase in RBC, Hb and PCV while decrease in MCV. There was a statistically significant improvement in the level of RBC and Hb

($p < 0.05$) with the treatment of TIM2 (200mg/kg). The improvement seen with the treatment of TIM2 was comparable with the standard drug. There was no further increase in the level of RBC and Hb with TIM3 (400mg/kg). This explains that the response to treatment with oral indiffusible mixture of *T. indica* leaf extract was dose related. TIM1 showed increase in level of RBC, Hb and PCV to a submaximal level when compared to TIM2. There were no much changes in MCH in all the groups including the standard whereas the MCV significantly reduced ($p < 0.05$) with the treatment of TIM2. However, short duration of present study is proposed to be a limitation, thereby longer duration is required for obtaining better responses.

CONCLUSION

It is postulated that the presence of flavonoids, phenols and iron in herbal extracts are responsible for the haematinic activity. The oral indiffusible mixture of *T. indica* leaf extract significantly increased the haemoglobin, and RBC count in anaemic rats indicating the haematinic activity at the dose of 200mg/kg. Thus, 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.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest

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TABLES

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 500ml		

TIM- *T.indica* Mixture

TABEL 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(mls^{-1})	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

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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

TABEL 3: Sedimentation volume of different formulations

Formulation	$F= V_U/ V_O$							
	1hr	2hr	6hr	12hr	1^{st}day	2^{nd} day	3^{rd}day	4^{th} 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

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 MotorActivity 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	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	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

Values are expressed as Mean ± SEM; n=6; [a]p< 0.001 in comparison with normal control; *p<0.05 in comparison with Disease control