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Hypotensive and Toxicological Study of Citric Acid and Other Constituents from *Tagetes patula* Roots

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Study of the effects of the methanolic extract of *Tagetes patula* roots on blood pressure led to the isolation of well known citric (**1**) and malic acid (**7**) as hypotensive, and pyridine hydrochloride (**4**) as hypertensive constituents of the plant along with a new constituent, 2-hydroxy, 5-hydroxymethyl furan (**9**). Citric acid and malic acid caused 71% and 43% fall in Mean Arterial Blood Pressure (MABP) of rats at the doses of 15 mg/kg and 30mg/kg respectively while pyridine hydrochloride produced 34% rise in the MABP of rats at the dose of 30mg/kg. LD_{50} and LD_{100} of citric acid in mice have been determined as 545 mg/kg and 1000 mg/kg, respectively.

Key words: *Tagetes patula*, Roots, Asteraceae (Compositae), Blood pressure, Toxicity

INTRODUCTION

Hypertension is a root cause for several serious disorders, like congestive heart failure, renal failure and cerebrovascular diseases. The disease exists so widely that about 10-20% of world population suffers with this syndrome. Side effects associated with most of antihypertensive drugs play an important role in the development of incurable complications. Hence, a need for the safe and effective drug with minimum or no side effects is still high. Keeping this in view as well as the medicinal value of indigenous plants, present paper describes the hypotensive and toxicological evaluation of *Tagetes patula*.

Tagetes patula (French marigold, Asteraceae), locally known as jafri, grows in gardens for ornaments across the globe and is used for the preparation of high grade perfumes in France (Chadha, 1976). The plant has been reported to possess hepatoprotective, diuretic (Vasilenko *et al.*, 1990), insecticidal (Wells *et al.*, 1993), fly repellant, antiseptic, blood purifying and nematocidal activities. Due to its antimicrobial activities, the plant is used to treat several dermatological disorders (Chadha, 1976; Kagan, 1991). Recently, flower petals of *T. patula* have been reported to possess anti-inflammatory activity (Kasahara, *et al.*, 2002). Pharmacological evaluation of chemical constituents isolated from *T. patula* showed that its flavonoids and their glycosides (Bhardwaj *et al.*, 1980; Ivancheva and Zdravkova, 1993) possess cholagogic activity (Koloshina, *et al.*, 1980); steroids and triterpenes (Kasprzyk and Kozierowska, 1966) are antimicrobial in nature (Vasudevan *et al.*, 1997); polyacetylenes (Kyo *et al.*, 1990) have cercaricidal activity (Towers *et al.*, 1984) while thiophene, its bi and trimers (Menelaou *et al.*, 1991) are responsible for nematocidal and insecticidal activities (Kyo *et al.*, 1990; Kagan, 1991). Helenien which is a dipalmatic acid ester of lutein, benefits retina (Tarpo and Cucu 1961). On the basis of its biologically active constituents, plant can be divided into two main parts i.e. aerial and subterranean. Aerial parts mostly contain biocidal terpenes while, subterranean part (root) is characterized by nematocidal thiophenes. Although plant is already used in the preparation of cardiovascular drugs (Grigorescu *et. al.*, 1987) yet no work on its hypotensive activity has been reported.

Current studies describe the detection of a new compound (**9**) along with well known citric acid (**1**) (Pouchert, vol. 1, 1983), its trimethyl (**2**) (Anagnostopoules, 1953) and dimethyl (**3**) (Hand Book, 1985) derivatives, pyridine hydrochloride (**4**) (Pouchert, 1983), 2,2',5',2"-terthiophene (**5**) (Buckingham, 1994; Kagan, 1991), dimethyl mallate (**6**), malic acid (**7**) (Buckingham, vol. 3, 1982) and 2-formyl, 5-hydroxymethyl furan (**8**) (Buckingham, 1982) from roots of

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T. patula. All these constituents except citric acid were obtained by methylation of the active fraction JRMI. Except thiophene, all the compounds detected here are new from the source and this is the first time that their effect on blood pressure has been measured. Prior to this investigation sodium citrate was once reported to cause prompt fall in blood pressure of cats at the dose of 30-45 mg/kg (Salant and Kleitman, 1922).

MATERIALS AND METHODS

Instrumentation

^{1}H-NMR spectra were obtained using a Bruker Aspect AM-400 and AM-500 spectrometer operating at 400 and 500 MHz. Solvent used were D_2O (**1**, **7**), $CDCl_3$ (**3**, **5**) and CD_3OD (**2**, **4**, **6**, **8**, and **9**). All chemical shifts were reported with respect to residual solvents signals. The EI and HR mass spectra were recorded on Finnigan MAT-112 and JMS HX-110 analysers. UV (in MeOH) and IR (in KBr, $CHCl_3$) spectra were obtained from Hitachi-U-3200 and JASCO-A 302 spectrophotometers respectively. Light petroleum refers to the fraction boiling in the range 66-70°C. Structures of known constituents were determined partly through spectroscopy and partly through comparison with the literature values (Hand Book, 1985; Pouchert, 1983; Buckingham, 1982, 1994). Purity of compounds was checked by Preparative Thin Layer Chromatography (PTLC) on silica gel 60 GF_{254} coated plates.

Plant material

Fresh, undried and uncrushed roots of *T. patula* were collected in the month of May 1998 from Karachi region, identified by plant taxonomist, Dr. Rubina Dawar, and a voucher specimen (KUH GH No. 67280) was deposited in the department of Botany, University of Karachi.

Extraction and isolation

Roots (3.75 kg) were extracted with petroleum ether (PE, 2.5 l) thrice at room temperature. The extracts were combined and freed of solvent in vacuum to afford a concentrated mass (JRP, 27.23 g). The marc was then percolated with methanol (MeOH, 2.5 L) thrice at room temperature. The methanolic extracts were combined and evaporated in vacuo to oily residue (JRM, 62.20 g). Addition of MeOH (40-50 mL) to JRM resulted in the separation of white precipitates (JRMI, 7.45 g) which turned brown during filtration. Filtrate obtained was evaporated to an oily residue (JRMF, 53.79 g). JRMI (2 g) was then subjected to Vacuum Liquid Chromatography (VLC) [Kieselgel 60 GF_{254} (6 g), ethyl acetate (EtOAc, 600 mL), MeOH (990 mL) and water (550 mL) in order of increasing polarity by 10 %]. The volume eluted with each system was 0.1 L which, afforded 30 fractions. Fraction No. 15 to 20 (EtOAc : MeOH, 8 : 2, 7 : 3, 6 : 4, 1 : 1, 4 : 6, and 3 : 7) gave white residue on evaporation which was characterized as citric acid [60.76 mg, mp 152-154°C, lit. (Pouchert, vol. 1, 1983), 153-154.5°C] on the basis of ^{1}H-NMR, IR, UV and MS spectroscopy as well as by the formation of its trimethyl (**2**) and dimethyl (**3**) derivatives.

Citric acid (1)

UV λ_{max} (MeOH) nm: 201.6 ; IR ν_{max} (KBr) cm^{-1}: 2950, 1711, 1425, 1395, 1363, 1241-1124 and 782; EIMS *m/z* (%) : 147 [M^+-COOH] (37); 142 [M^+-3xH_2O] (3.0); 138 (14.3), 130 (16.9), 129 (87.3), 115 (13.7), 112 (71.0), 102 (82.1), 91 (11.0), 87 (86.5), 85 (32.1), 84 (100), 69 (75.2), 68 (48.7), 67 (24.5), 61 (15.2), 60 (88.7), and 56 (86.51); ^{1}H-NMR (D_2O, 500 M) δ_H: 2.55 (d, $J_{2a,2b=4a,4b}$ 16.70, H-2a, H-4a) and 2.39 (d, $J_{2b,2a=4b,4a}$ 16.75, H-2b, H-4b).

Methylation of citric acid

Citric acid (20 mg) was treated with methanolic hydrochloric acid (15 mL) for two days at room temperature. A white solid mass was deposited at the bottom of flask, which was extracted with PE (20 mL) and PE : EtOAc (1:1, 50 mL) to give trimethyl citrate (**2**) [5.2 mg, mp 79-80°C, (lit. mp, 76-80°C, Hand Book, 1985] and dimethyl citrate (**3**) [9.8 mg, white needles (PE : EtOAc 1:1), mp 102-105°C]. Structures of trimethyl citrate (**2**) [Hand Book, 1985] and dimethyl citrate (**3**) (Anagnostopoulos, 1953) were elucidated through detailed spectral studies.

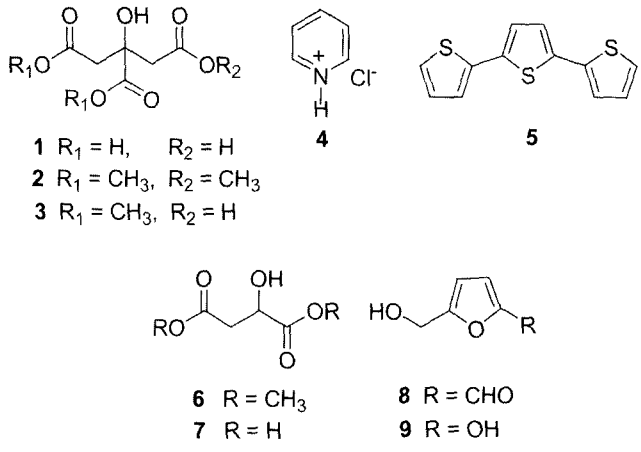
Trimethyl citrate (2)

UV λ_{max} (MeOH) nm: 204.2; IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 2952, 1742, 1443, 1325, 1215 and 1124; EIMS *m/z* (%) : 235 (MH^+; 3.68); 175.0611 (M^+-59, $C_7H_{11}O_5$; 51.87); 143.0328 ($C_6H_7O_4$; 100); 111.0072 ($C_5H_3O_3$; 32.0); 101.0220 ($C_4H_5O_3$; 78); and 59 (51); ^{1}H-NMR (CD_3OD, 500 M) δ_H: 2.88 (d, $J_{2a,2b\ =\ 4a,4b}$ 15.65, H-2a, H-4a) 2.79 (d, $J_{2b,2a\ =\ 4b,4a}$ 15.60, H-2b, H-4b), 4.10 (s, exchangeable with D_2O, 3-OH), 3.67 (s, 1,5-CO_2CH_3) and 3.81 (s, 3-CO_2CH_3).

Dimethyl citrate (3)

UV λ_{max} (MeOH) nm: 204.1; IR ν_{max} ($CHCl_3$) cm^{-1}: 3455, 2953, 1740, 1440, 1245, 1225 and 1187; EIMS *m/z* (%) : 221 (MH^+; 2.50); 175 (30.31), 171 (29.53), 161 (56.09), 144 (38.49), 143 (100.00), 139 (38.07), 129 (57.75), 116 (36.19), 115 (10.38), 111 (58.11), 102 (21.40), 101 (78.86), 74 (50.64), 69 (53.75), 60 (23.66), and 59 (62.57); ^{1}H-NMR ($CDCl_3$, 500 M) δ_H: 2.88 (d, $J_{2a,2b}$ 15.7, H-2a), 2.79 (d, $J_{2b,2a}$ 15.7, H-2b), 2.92 (d, $J_{4a,4b}$ 16.0, H-4a), 2.83 (d, $J_{4b,4a}$ 16.0, H-4b), 5.31 (br.s, exchangeable with D_2O, 3-OH), and 3.67 (s, 1-5-CO_2CH_3).

Rest of the VLC fractions of JRMI showing either tailing or immovable pink spot at the bottom of TLC plate,



1 R_1 = H, R_2 = H
2 R_1 = CH_3, R_2 = CH_3
3 R_1 = CH_3, R_2 = H

4 **5**

6 R = CH_3 **8** R = CHO
7 R = H **9** R = OH

suggested the presence of highly polar constituents. Hence for the purpose of isolation of active constituents, fraction JRMI was methylated. Earlier some fractions from *T. patula* were acetylated for the isolation of quercetagetin derivatives (Bhardwaj *et al.*, 1980).

Methylation of fraction JRMI

Fraction JRMI (1.95 g) was treated with methanolic hydrochloride (15 mL, room temperature, overnight). Residue (JRMIM, 2.34 g) obtained on evaporation of reaction mixture, was fractionated with different solvents including, PE (70 mL), EtOAc (100 mL) and MeOH (80 mL). Ten fractions thus obtained (volume of each fraction was 25 mL) were evaporated under hood to give residues E-1--E-10. Residue E-1 (PE, 15.3 mg), E-3 (PE : EtOAc, 1:1; 32.4 mg) and E-8 (EtOAc : MeOH, 1:2; 33.4 mg) were identified as trimethyl citrate (**2**), dimethyl citrate (**3**) and pyridine hydrochloride [**4**, δH, 8.91m, H-2,6; 8.13 br.t. (7.1) H-3,5; 8.66t (8.4), H-4] respectively through detailed spectroscopy (^{1}H-NMR, ^{13}C-NMR, MS, UV and IR). Fraction E-4 (PE : EtOAc, 1:2; 21.2 mg), E-5 (EtOAc, 29.3 mg), E-6 (EtOAc : MeOH, 2:1; 42.4 mg) and E-7 (EtOAc : MeOH, 1:1; 53.4 mg) were further purified through PTLC. Thin layer Chromatography of E-4 (PE : EtOAc, 8:2), E-5 ($CHCl_3$: MeOH, 9.5 : 0.5) and E-7 ($CHCl_3$: MeOH, 9.5 : 0.5) afforded pure 2,2',5',2"-terthiophene (**5**, R_f 0.56, PE; 9.81 mg), dimethyl mallate (**6**, 7.3 mg) and malic acid (**7**, R_f 0.52, MeOH; 26.23 mg) respectively.

^{1}H-NMR of E-6 revealed the presence of two compounds, 2-formyl, 5-hydroxymethyl furan [**8**, δ_H 7.71 d (4.3) H-3; 6.59 d (4.3) H-4; 4.61 s, $\underline{CH_2}OH$; 9.53 s, CHO] and 2-Hydroxy, 5-hydroxymethyl furan [**9**, δ_H 6.27 d (3.9) H-3; 6.35 d (3.9) H-4; 4.49 s, $\underline{CH_2}OH$] in a ratio of 2:1. PTLC of E-6 ($CHCl_3$: MeOH, 9.5 : 0.5, 21.5 mg) also evidenced two pink bands with R_f value of 0.42 (**8**) and 0.33 (**9**). Pure compounds obtained after PTLC were so small in quantity that no spectra could be taken. Both compounds thus identified in a mixture form.

Animals and drugs

Animals used in this study were Sprague Dawley rats (200-250 g) and NMR-1 mice (25-30 g). They were housed at the Animal House of Dr. HMI Institute of Pharmacology and Herbal Science, Hamdard University at 28°C and were given a standard diet and tap water *ad libitum*. Drugs used were acetylcholine and sodium chloride from E. Merck, atropine sulfate from Boehringer Ingelheim and pentothal® sodium from Abbott Karachi. All these drugs were dissolved in distilled water. Acetylcholine (10^{-6} M) and saline (0.9% NaCl) were used as positive and negative controls respectively.

Hypotensive activity

Normotensive Sprague-Dawley rats (200-250 g) were anaesthetized with sodium pentothal (50 mg/kg i.p.). The trachea was exposed and cannulated to facilitate spontaneous respiration. Drugs were injected (vol. 0.2 mL) through a polyethylene cannula inserted into the external jugular vein followed by a saline flush (0.2 mL). The arterial blood pressure was recorded at 10 a.m. to 2 p.m., from the carotid artery via polyethylene arterial cannula connected to a Research Grade Blood Pressure Transducer (Harvard, 60-3003) coupled with four channel Harvard Universal Oscillograph (Curvilinear, 50-9307). The temperature of the animal was maintained at 37°C by use of overhead lamp. Animals were allowed to equilibrate for at least 15 min before administration of any drug. Mean Arterial Blood Pressure (MABP) was calculated as sum of the diastolic blood pressure plus one-third pulse width. Changes in blood pressure were expressed as the percent of control values, obtained immediately before the administration of test substance (Saleem, 1999).

Toxicity evaluation

Different groups of mice with either sex (six animals per group) were used to measure the acute toxicity of JRMI and citric acid by intraperitoneal route of administration. Group-1 treated as control, was given saline (0.5 mL/mice) while group-II, III and IV were treated with 0.1 g/kg, 1.0 g/kg, and 3.0 g/kg of JRMI. Group-V, VI and VII were injected with 0.1 g/kg, 0.5 g/kg and 1.0 g/kg of citric acid. All the animals were treated daily for seven consecutive days and kept under constant observation for two hours after dosing to observe any change in general behavior or other physiological activities and weighed daily on electronic balance to monitor any change in body weight. LD_{50} was calculated by Reed and Muench method (Bancroft, 1966).

Statistical analysis

Changes in blood pressure were compared using analysis of variance followed by students *t*-test. Values of

$P<0.05$, $P<0.01$, and $P<0.001$ were considered to be significant.

RESULTS AND DISCUSSION

Methylation of JRMI led to a residue, which after extraction with different solvents and PTLC (*vide* Material and Methods) gave new furan derivative (**9**). Because of the paucity of compound, no spectra could be obtained. However, in its mixture form (E-6), it exhibited molecular ion peak at *m/z* 114.0356 corresponding to molecular formula $C_5H_6O_3$ in HREIMS. Measurement of integration in ^{1}H-NMR spectrum of E-6 suggested, that two components (**8** and **9**) exist in a ratio of 2:1 respectively.

The ^{1}H-NMR spectrum of E-6 in MeOH showed a pair of doublet at δ 6.27 and δ 6.35 (J = 3.9 Hz, H-3 and H-4 respectively) for aromatic protons while, a singlet resonated at δ 4.49 attributed to hydroxymethane. Rest of the signals in the spectrum were exactly comparable to the published data of 2-formyl, 5-hydroxymethyl furan (**8**). Upfield shift of H-3 at δ 6.27 as compared to that of 2-formyl-5-hydroxymethyl furan at δ 7.41 suggested the presence of hydroxyl group at C-2 in compound **9**. Mass fragments at *m/z* 96 (M^+-18), 83 (M^+-31) and 65 (M^+-31, -18) confirmed the structure of compound **9** as 2-hdroxy, 5-hydroxymethyl furan.

The mass spectrum of citric acid (**1**) did not show the molecular ion peak which is consistent with the reported data (NIST, 1992). The ^{1}H-NMR spectrum in D_2O displayed a pair of two-proton doublet (J=16.7 Hz) at δ 2.39 and 2.55 for symmetrical pair of methylene protons while literature reported a sharp singlet for this molecule at δ 2.7 in DMSO-d_6 (Pouchert, 1983). Sharpness of signal for 3-hydroxyl proton (exchangeable with D_2O) in ^{1}H-NMR and IR spectra (*vide* experimental) of compound **2** as well as broadness of same signal in compound **3** due to more hydrogen bonding, rightly justified the elucidation of structures for trimethyl and dimethyl citrates.

Hypotensive activity

Fats free methanolic extract of *T. patula* roots (JRM) and its fractions, JRMI and JRMF showed comparable effect on MABP of rats in a dose dependent manner. They caused 17-21% and 32-35% fall in MABP at the corresponding doses of 3 mg/kg and 30 mg/kg (Table I).

VLC of JRMI afforded citric acid (**1**). Citric acid, which is present in almost all plants as well as in many animal tissues and fluids is the main constituent of genus citrus (family Rutaceae) and was first isolated in 1784 from lemon juice (The New Encyclopaedia, 1975). Beside, its use as a flavouring agent in confections and soft drinks (The New Encyclopaedia, 1975), citric acid has also been utilized in the preparation of antioxidant (Armando *et al.*, 1998), anticoagulant (Schneider *et al.*, 1997) and anti-diabetic (Bywater *et al.*, 1991) formulations. In the present studies, citric acid has reduced blood pressure of normotensive rats in a dose dependent manner. It caused 23% and 71% decrease in MABP at the doses of 3 mg/kg and 15 mg/kg (Table I) respectively. Its dimethyl derivative (**3**) did not show any significant decrease at 3 mg/kg, however, at the dose of 30 mg/kg, 34% reduction in blood pressure was recorded (Table I). It is worth mentioning here that, without giving % of fall or any other detail, literature describes the hypotensive activity of sodium salt

Table I. Effects of Extract/Fractions/Pure compounds from *Tagetes patula* root on Mean Arterial Blood Pressure (MABP) of normotensive rats

Name	Dose mg/kg	MABP Before Treatment (mmHg ± SEM)	MABP After Treatment (mmHg ± SEM)	% Change in MABP (mmHg)	Duration (sec. ± SEM)
JRM[a]	3	141.30 ± 14.61	113.5 ± 14.13	-19.67*	14.4 ± 5.13
	30	126.54 ± 10.64	86.19 ± 7.95	-31.89**	24.25 ± 10.76
JRMI[b]	3	114.66 ± 6.24	95.16 ± 9.24	-17.01***	10.66 ± 5.67
	30	134.74 ± 6.13	90.92 ± 8.20	-32.52**	35.82 ± 16.56
JRMF[c]	3	138.89 ± 4.61	110.09 ± 15.3	-20.73*	12.4 ± 5.19
	30	129.56 ± 11.94	85.17 ± 15.73	-34.81***	12.6 ± 6.95
Citric acid (1)	3	124.39 ± 5.33	95.30 ± 3.68	-23.38**	60.57 ± 11.27
	15	137.42 ± 16.92	39.19 ± 11.18	-71.48**	55.16 ± 15.11
Dimethyl citrate (3)	30	128.27 ± 10.36	84.44 ± 11.23	-34.02***	20.6 ± 4.66
Pyridine hydrochloride (4)	30	92.16 ± 7.59	123.18 ± 8.65	+33.66**	78 ± 9.57
Malic acid (7)	30	117.67 ± 8.19	68.5 ± 10.23	-41.78**	21.76 ± 3.46

Each value shown represents Mean ± SEM (Standard Error Mean) of six determinations; [a]Fatty acid free methanolic extract of roots; [b]Precipitate obtained from JRM; [c]Oily residue from filtrate of JRM ;¯ % Decrease in MABP; +% Increase in MABP; * $P<0.05$; ** $P<0.001$; *** $P<0.01$.

of citric acid in cats at the dose of 30-45 mg/kg (Salant *et al.*, 1922).

Pyridine hydrochloride (**4**) which is an antidepressant, antiseptic, antiamoebic, bactericidal and herbicidal compound and has also been used as an intermediate in the preparation of sulfadrugs (Abramovtich, 1968), showed hypertensive effect unlike to that of citric acid (**1**) and dimethyl citrate (**3**). It caused 34% rise in MABP of rat at 30 mg/kg (Table I). Malic acid (**7**) which is used as a food additive (Buckingham, 1982) and occurs predominantly in various fruits as flavouring agent (Irwin, 1967) exhibited 41% decline in MABP at the dose of 30 mg/kg. All the changes observed in MABP are significant (Table I). Activity of JRP, JRMIM and compounds, trimethyl citrate (**2**), 2,2',5',2''-terthiophene (**5**) and dimethyl mallate (**6**) could not be determined due to solubility problem.

Duration of hypotensive action for JRM, JRMI, JRMF, dimethyl citrate (**3**) and malic acid (**7**) was extremely brief and lasted for less than one minute (Table I). Citric acid, however, showed its effect for one minute at both doses while hypertension due to pyridine hydrochloride lasted for more than a minute (Table I). None of these substances show any change in heart rate of rats (330 ± 30 bpm), however, in case of citric acid (**1**), at the higher dose of 15 mg/kg heart rate was dropped to 120 ± 30 bpm from 330 ± 30 bpm for a very brief period of 6 seconds and then returned to normal value.

All the substances except JRM showed similar potency of hypotensive action in rats pretreated with atropine sulfate (10^{-4} M/kg) as was observed in non-atropinized animals. It may therefore be assumed that mode of action of these substances is different from that of acetylcholine and muscarinic receptors M_2 on heart muscles are not involved in lowering the blood pressure. Literature, however, revealed that cats treated with atropine and pilocarpine revert the hypotensive action of sodium citrate (Salant and Kleitman, 1923). Hypotensive effect of JRM in atropinized rats (at 30 mg/kg) was reduced to 50% (MABP decreased from 125.31 mmHg to 105.27 mmHg, %fall ~16%), indicating that in addition to the constituents that may act through non-cholinergic receptors, muscarinic receptor mediating principles may also be present in crude extract.

Toxicological studies

Intraperitoneal administration of JRMI did not show any change in physical behaviour of mice at the dose of 0.1 g/kg while, 1 g/kg appeared as LD_{50} and out of six, three mice died at the interval of 24 h. At the dose of 3 g/kg all mice died in a period of 10 min-24 h.

Citric acid caused significant loss of weight with the expiry of one mice on intraperitoneal administration of 0.1 g/kg. A lot of symptoms like, dyspnea, increased respiratory depth, tremors, convulsions, and tail erection were observed for more than an hour at the dose of 0.5 g/kg. Later on, two mice died on fourth day of dosing, and rest did not exhibit any change in physical activity. Lethal dose was determined as 1 g/kg, as all mice died in a period of 15 min-3 days. Before death, some of these mice showed symptoms similar to those observed at the dose of 0.5 g/kg. LD_{50} calculated by Reed-Muench method (Bancroft, 1996) was 545 mg/kg. Literature, however, reported 5 mM/kg or 960 mg/kg of citric acid as LD_{50} (Gruber and Halbeisen, 1948). Difference with literature value may be explained on the basis of strain of mice. In present case NMRI-I mice were used while in case of reported value strain of mice is not known.

CONCLUSION

Tagetes patula, an ornamental plant, which is commercially employed in perfume industry, has significant medicinal value. Current bioassay directed analysis of plant has discovered the presence of constituents that may affect the blood pressure. Citric acid, dimethyl citrate (obtained as an artifact) and malic acid have been detected as hypotensive principles while pyridine hydrochloride as hypertensive constituent of root. Citric acid, which has emerged as the most potent hypotensive agent in current study, also seems to be more toxic, as its LD_{50} and LD_{100} are comparatively smaller than JRMI. This looks strange as citric acid is the main component of commonly eaten citrus fruit. The difference of observation can be explained on the basis of route of administration. Through intraperitoneal route, citric acid may cause toxicity due to possible acidosis in blood. While, through oral intake (in crude form of citrus fruit), it may enter Kreb cycle and produces ATP in mitochondria, that generates energy, hence intake is safe and beneficial. However, for safety evaluation of pure citric acid through oral route as well as development of more potent and safe hypotensive agents from *T. patula,* more work is required.

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