Bangladesh J. Bot. **50**(1): 21-28, 2021 (March)

IN VITRO AND *IN VIVO* BIOLOGICAL APPROACH TO VALIDATE FOLKLORIC CLAIMS OF *TRIANTHEMA TRIQUETRA* ROTTLER & WILLD

FATIMA SAQIB, MEHWISH SHAUKAT, SANA JAVAID[1], MUHAMMAD RIAZ*[2], ZAHID KHAN[3], CALIN COBELSCHI[4], DANA FESTILA[4] AND CRISTINA NICULA[5]

Department of Pharmacology, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

Keywords: Biological approach, *Trianthema triquetra*, Validate folkloric claims

Abstract

Trianthema triquetra Rottler & Willd (Tt.Cr) is used in traditional practices as a remedy for various ailments. Hence current research was commenced to authenticate the folkloric uses. To discover spasmolytic potential, Tt.Cr was applied to isolate jejunum, while isolated tracheal and aorta tissues were used to determine the tissue relaxing properties of the extract. Anti-lipoxygenase activity was determined *in vitro* using Baicalein as standard. *In vivo* testing was carried to examine the potentiality of the herb to treat pyrexia and pain. Tt.Cr showed dose-dependent (0.01 - 3.0 mg/ml) spasmolytic effects in jejunum tissues and relaxed K^+ (80 mM)-induced spasm and triggered rightwards shift of Ca^{+2} concentration-response curves. Carbachol (1μM)- together with K^+ (80 mM) - induced tracheal spasm was also relaxed by Tt.Cr (0.01 to 1.0 mg/ml). Additionally, Tt.Cr (0.01 - 1.0 mg/ml) relaxed phenylephrine (1 μM) and K^+ (80 mM) - treated constricted rabbit aorta. Tt.Cr (0.5 mM) inhibited lipoxygenase enzyme. Tt.Cr (80 mg/kg) settled pyrexia in rabbits comparable to aspirin and prolonged tail deflection time in mice (100 mg/kg) hence proving analgesic activity. The Tt.Cr demonstrated antispasmodic, bronchodilation and vasodilation properties probably by blocking calcium channels. These outcomes generate logic behind ancient application of herb for numerous ailments such as asthma, cough, heart problems and spasm.

Introduction

Trianthema triquetra (Aizoaceae) is commonly known as Alettie, Chulani (Ahmad *et al.* 2015), Choti Ulwaiti (Ahmad *et al*. 2014), Loonaki in Pakistan (Zarin *et al*. 2016), Naayi Soppo, Lunki, Lutanki in India, red spinach, small hogweed in Australia (Quattrocchi 2012). Plant paste has been applied on swellings by population for rheumatism (Katewa and Galav 2005) and possessed anti-inflammatory potential both *in vitro* and *in vivo* bioactivity test (Sathyaraj and Indumathy 2018). It exhibited hepatoprotective effects when investigated on rats (Chitra and Nithyanandhi 2007) and its roots showed significant gastric anti-secretory and acid neutralizing effects (Ghori 2016). The plant can cure cold, flu, cough and asthma (Akbar and Khatoon 2012, Ahmad *et al*. 2015) thus rendering its tendency towards its potential as an antitussive, bronchodilator and antipyretic but as it has not been searched for these activities. Chemically plant comprises thiobarbituric acid, reduced glutathione, glutathione peroxidase, and catalase (Chitra and Nithyanandhi 2007). Preliminary phytochemical testing (Tona *et al.* 1998) revealed that plant extract possesses saponins, tannins, and flavonoids.

*Author for correspondence: <pharmariaz@gmail.com>. [1]Department of Pharmacy, The Women University Multan, Pakistan. [2]Department of Pharmacy, Shaheed Benazir Bhutto University, Sheringal, Pakistan. [3]Faculty of Pharmacy, Federal Urdu University of Science and Technology, Karachi Pakistan. [4]Faculty of Medicine, Transilvania University of Brasov, Romania. [5]Faculty of Medicine, Iuliu Hatieganu, University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Irregular contractions of smooth muscles in GIT, respiratory tract or cardiovascular system results in diarrhoea, asthma and hypertension. Cytosolic Ca^{+2} plays a vital part in the functioning of smooth muscle and calcium exposure of contractive components alters depending on the milieu neighbouring the cell (Karaki *et al.* 1997). For inspection of probable smooth muscle relaxant effects of a plant extract, isolated organs from a rabbit might be deployed, as rabbits illustrate numerous structural and functional resemblances of human organs. Saqib *et al.* (2019) did investigations using isolated tissues of different animals.

Materials and Methods

The whole herb of *Trianthema triquetra* was collected fresh from BZU Multan, Pakistan, during August 2011 and Dr. Altaf A. Dasti (taxonomist) identified the plant and Voucher No. (P.Fl.235-4) was retained there. Fresh herb (1 kg) was subjected to shade drying followed by coarse grinding. Coarse powder was macerated in 80% methanol-aqueous for one week followed by filtration and whole technique was repeated twice. Combined extracts were subjected to evaporate on rotary evaporator resulting brownish extract which was stored at –20°C. On day of experimentation, fresh dilutions of extract were prepared (Saqib *et al.* 2018).

All the chemicals, solvents, and drugs used were of reagent analytical grade. Acetylcholine, carbachol, verapamil chloride and potassium chloride were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Methanol, EDTA, glucose, magnesium chloride, magnesium sulfate, phenylephrine, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and calcium chloride were purchased from Merck, Darmstadt, Germany. Sodium chloride, ammonium hydroxide and sodium hydroxide were obtained from BDH laboratory supplies, Poole, England.

For studies on isolated organs, adult rabbits weighing between 1.0 and 1.5 kg of either sex were used. In *in vivo* studies, albino rabbits and mice were used as animal model to find out antipyretic and analgesic potential of plant. All animals accommodated properly in animal house were normally fed with standard diet and water and were starved a night before experiment. Entire experimentations were accomplished by fulfilling instructions of the Institute of Laboratory Animals Resources, Commission on Life Sciences (Council 2010) and by getting authorization from Ethical Committee of the BZU Multan (EC/03/2011 dated 16.02.2011).

Tissues were prepared in laboratory and were subjected to Tt.Cr in accordance to the already reported method (Saqib and Janbaz 2016). Involvement of CCB phenomenon for antispasmodic characteristics was investigated by method and verified by developing CRCs using tyrode rich (Farre *et al.* 1991).

Tissues were prepared in laboratory and were evaluated for bronchodilator action according to the method by Saqib *et al.* (2019). Tissues were also prepared in laboratory and were evaluated for vasodilator action according to described method (Saqib *et al.* 2015). Lipoxygenase inhibiting potential of Tt.Cr was explored according to the technique (Tappel 1953) with little adjustments. Pyrexia resolving characteristics of Tt.Cr was evaluated by using albino rabbits according to cited technique (Khan *et al.* 2008). Characteristics of Tt.Cr in depressing nociception were evaluated by using albino mice according to cited technique (Asongalem *et al.* 2004, Adedapo *et al.* 2008).

Facts and figures were articulated as the mean ± SEM. EC_{50} values along with 95% CI were calculated by using the computer software "Graph pad Prism Program" (version 5.0), San Diego CA, USA. Concentration response curves were analysed by the non-linear regression of sigmoid response curve (variable slope). The statistic applied was the students t-test and $p < 0.05$ was considered quite noteworthy.

Results and Discussion

Addition of Tt.Cr to jejunum tissues yielded relaxation in a cumulative dose dependant manner (Fig. 1a) with EC_{50} of 0.12 mg/ml. (95% CI: 0.01 - 0.28; n = 5). It induced prominent relaxation of high K^+ contracted tissues (Figs 1b & 2a) with EC_{50} of 0.20 mg/ml. (95% CI: 0.01 - 0.61; n = 5). These outcomes were analogous to the effects of verapamil used as standard drug and relaxed both spontaneously contracting and high-K treated jejunum (Fig. 2b) with EC_{50} value of 0.65 µM (95% CI:0.5551 - 0.8601, n = 5) and 0.70 µM (95% CI:0.5777 - 0.8881; n = 5), respectively.

The capability of Tt.Cr to block calcium channels was additionally verified on Calcium response curves (CRCs). The Ca^{+2} free media fully exterminated the spontaneous constrictions of tissues but on adding Ca^{+2} in a cumulative manner (0.1 - 6.4 mM), restoration of tissue contractions was obtained progressively, and best contraction was made at 6.4 mM concentration of Ca^{+2}. Afterwards, the jejunum tissue incubation with Tt.Cr (0.1 - 0.3 mg/ml) promoted the rightwards shifting of CRCs, and these effects were found equivalent to those yielded by verapamil (Fig. 3).

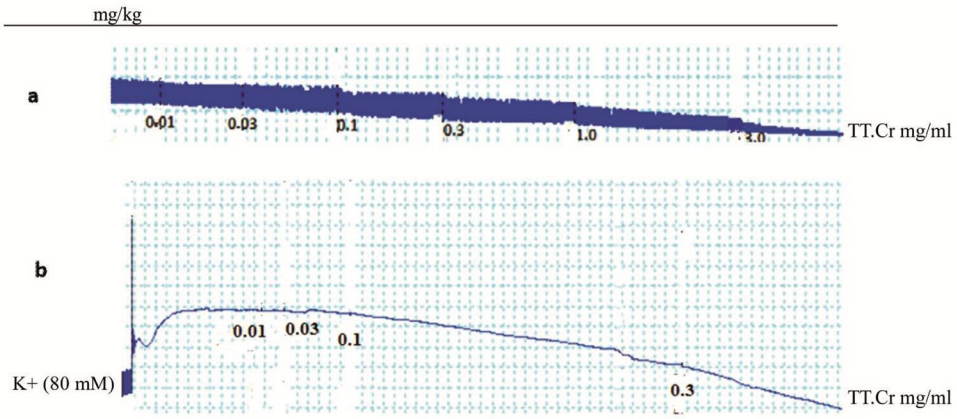

Fig. 1. Tracings showing (a) the relaxant effect of a crude methanolic extract of *Trianthema triquetra* (Tt.Cr) on spontaneous and (b) high K+ (80 mM)-induced tissue contraction. Tt.Cr was added at increasing concentrations and values listed were the final tissue bath concentrations (n = 5).

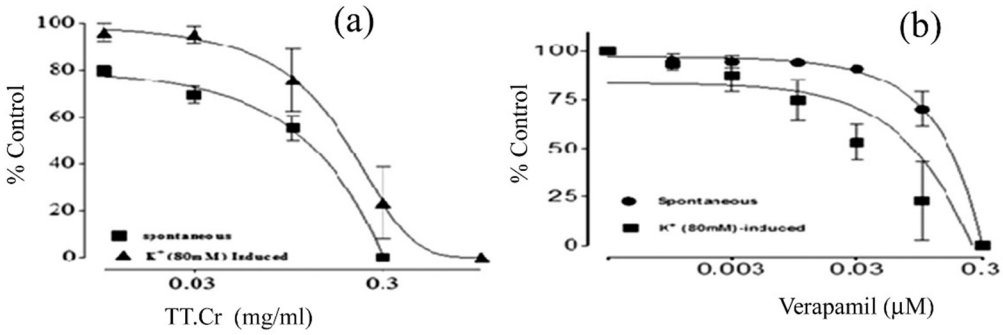

Fig. 2. Spasmolytic effects induced in concentration-dependent manner by (a) a methanolic extract of *Trianthema triquetra* (Tt.Cr) and (b) verapamil on spontaneous- and high K+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values are the mean ± SEM, n = 5.

Normal tracheal tissue remained unaffected by adding Tt.Cr. But cumulative dosing of extract on 80mMol KCl treated tracheal tissue, relaxation resulted (Fig: 4a) with EC_{50} of 0.14 (95% CI: 0.02 - 0.28; n = 5). Further testing on carbachol treated tracheal preparation, again tissue dilation was obtained (Fig. 4b) with EC_{50} 0.31 (95% CI: 0.01 - 2.20; n = 5) showing bronchodilator potential of plant. Likewise, verapamil also relaxed carbachol (1 µM) - and K^+ (80 mM) - exposed tracheal preparations with EC_{50} of 0.114 (95% CI: 0.057 - 0.227 mg/ml; n = 5) and 0.048 µM (95 % CI: 0.028 - 0.083 mg/ml; n = 5), respectively (Fig. 4c, d).

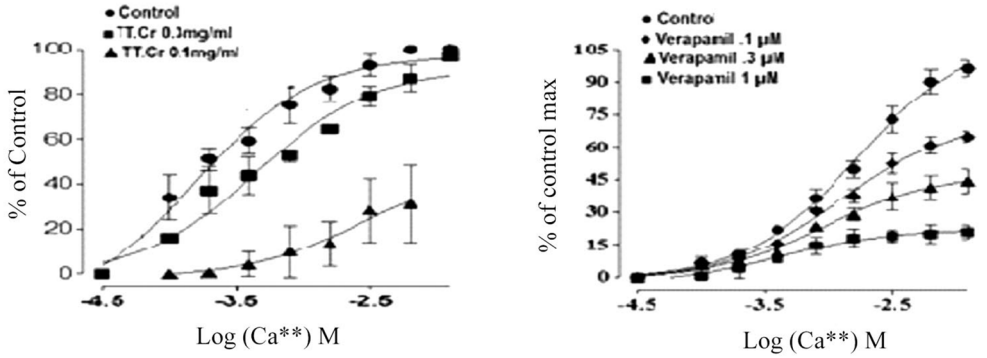

Fig. 3. Calcium antagonizing effect of (a) a methanolic extract of *Trianthema triquetra* (Tt.Cr) and (b) verapamil on concentration response curves of Ca^{+2}. in isolated rabbit jejunum preparations. Values are the mean ± SEM, n = 5.

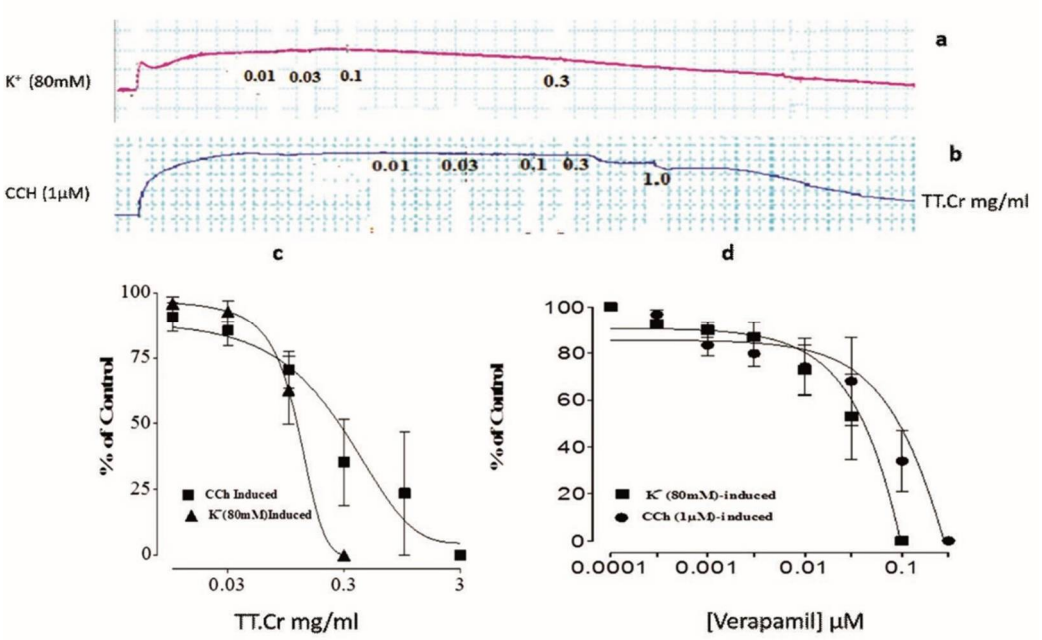

Fig. 4. Concentration dependent bronchodilator effect of a methanolic extract of *Trianthema triquetra* (Tt.Cr) (a, b and c) and (d) verapamil on carbachol (CCh: 1 µM)- and high K^+ (80 mM)- induced contractions in isolated rabbit tracheal preparations. Values are the mean ± SEM, n = 5.

Plant extract produced vasodilator effects when tested on high K^+ (Fig. 5a) and phenylephrine (Fig. 5b) treated constricting aortic tissues with EC_{50} of 1.74 mg/ml. (95% CI: 0.10 - 3.0; n = 5) and 0.24 mg/ml (95% CI: 0.01 - 0.82; n = 5) correspondingly. Similarly, verapamil too demonstrated vasodilation on phenylephrine (1 μ M) - and K^+ (80 mM) treated aortic tissues, with EC_{50} of 0.90 μM (95% CI: 0.03 - 5.02 μM; n = 5) and 0.43 μM (95% CI: 0.03 - 1.98 μM; n = 5), respectively (Fig. 5c, d).

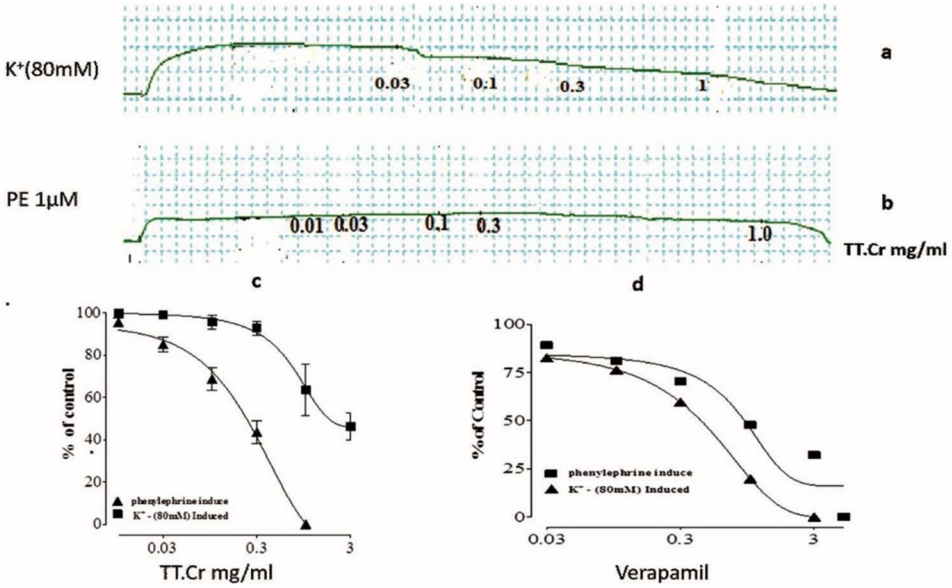

Fig. 5. Concentration dependent vasodilator effect of a methanolic extract of *Trianthema triquetra* (Tt.Cr) (a, b and c) and (d) verapamil on phenylephrine (PE: 1 μM)- and high K^+ (80 mM) induced contractions in isolated rabbit aorta preparations. Values are the mean ± SEM, n = 5.

T. triquetra caused *in vitro* lipoxygenase inhibition thus showing activity up to 50 ± 1.23% analogous to baicalein 93.79 ± 1.27% (Table 1).

Tt.Cr (80 mg/kg; i.p) significantly resolved pyrexia comparable with Aspirin hence signifying a probable antipyretic potential of Tt.Cr (Table 2).

Tt.Cr (75 mg/kg showed significant ($p < 0.01$) expansion of tail deflection time which was comparable with the effects of aspirin (Table 3).

In current study, DRCs were constructed to assess how *Trianthema triquetra* relaxed smooth muscles. Seeing that free Ca^{+2} in cytoplasm promotes smooth muscles contractions as evidenced by acetylcholine, histamine, and serotonin (Karaki *et al.* 1997). There might be an opening of L-type calcium or discharged from intracellular reservoirs (Van-Rossum 1963). Uninterrupted depolarization and repolarization induce rhythmic contractions of jejunum and inwards Ca^{+2} movements by L-type Ca^{+2} channels lead to depolarization (Bolton 1979). So, it may be claimed that by stopping either calcium influx or hindering calcium release from reservoirs within cells may stop the impulsive contractions in jejunum.

To understand the underlying mechanism, 80 mM KCL was applied to spontaneously contracting jejunum which led to persistent contraction due to calcium influx thus depolarizing the cell membrane and resulting in generation of smooth muscle spam (Hill-Eubanks *et al.* 2011).

Tt.Cr relaxed the contracted tissues at even lower dose. So it may be proposed that plant possesses calcium channel blocking potential (Karaki *et al.* 1997). It was further confirmed when extract shifted CRCs towards right on jejunum preparation as verapamil does (Bolton 1979).

Folkloric use of Tt.Cr in cough was reported by Akbar and Khatoon (2012) and CCBs are well known to resolve cough (Saqib and Janbaz 2016). Mostly, spasmolytic components are absorbed due to their non-polar and lipophilic nature and produce systemic effects thus acting on trachea (Saqib *et al.* 2015). Tt.Cr relaxed carbachol (1 μM) - and K+ (80 mM) treated constricted tracheal tissues in dose dependent manner and it is supposed by mediating Ca^{+2} channel blocking action (Bolton 1979).

Table 1. Inhibitory activity of Tt.Cr on lipoxygenase enzyme in comparison to standard.

Enzyme	Compound under study	Conc./well	% inhibition	IC_{50}
Lipoxy-genase	Tt.Cr	0.5 mM	50 ±1.23	0.34 ± 0.06 mg/ml
	Baicalin	0.25 mM	93.79 ± 1.38	0.13 ± 0.015 mM

Table 2. Effect of Tt. Cr on pyrogen-induced pyrexia in rabbit.

Groups	Dose	Tail deflection before treatment (sec)	Tail deflection after treatment (sec)
Control	Saline	2 ± 0.2	2.5 ± 0.6
Aspirin	75 mg/kg	2.5 ± 0.34	3.86 ± 0.14
Tt.Cr	100 mg/kg	2.3 ± 0.24	5.1 ± 0.25

Table 3. Effect of Tt. Cr and comparison with aspirin on tail deflection time (s) in mice.

Groups	Dose	Rectal temperature (hrs)				
		0	2	3	4	5
Control	Pyrogen + saline	38.62 ± 0.08	40.61 ± 0.02	40.15 ± 0.06	39.66 ± 0.16	39.54 ± 0.10
Aspirin	Pyrogen + aspirin 10 mg/kg	38.75 ± 0.08	40.82 ± 0.36	39.02 ± 0.11	38.95 ± 0.05	38.86 ± 0.05
Tt.Cr	Pyrogen + Tt.Cr 80 mg/kg	38.64±0.45	40.78 ± 0.06	39.99 ± 0.03	39.82 ± 0.04	38.73 ± 0.12

CCBs are famous for settling down cardiovascular issues, Tt.Cr was applied to rabbit aortic tissues contracted with phenylephrine- and K+ (80 mM) to elucidate the mechanism of calcium channel blocking activity (Saqib and Janbaz 2016). Tt.Cr displayed vasodilation when added on K^{+} (80 mM) - treated aortic tissues by Ca^{+2} channels blockade plus inhibition of Ca^{+2} discharge by endoplasmic reticulum. Phenylephrine-exposed aorta was relaxed too due to inactivation of α-adrenergic receptors and consequent no Ca^{+2} influx (Saqib *et al.* 2018).

Furthermore, Tt.Cr when examined *in vitro* led to inhibition of Lipoxygenase enzyme thus interfering the conversion of arachidonic acid into leukotrienes (Schaible *et al.* 2013) which in turn are well-known as constrictor of respiratory tract and also play role in inflammation. Hence it may be hypothesised that *T. triquetra* may prove useful in management of bronchoconstriction in asthma and inflammation as well as increased mucus secretion during allergic rhinitis.

The Tt.Cr demonstrated antipyretic and analgesic effects when tested in animal models. Prostaglandin plays vital role in inducing pyrexia (Loux *et al.* 1972) and perception of pain (Geusens *et al.* 2013). The prostaglandins are products of enzymes COX-1 and COX-2 on ω-3 and

ω-6 C20 fatty acids (Lone and Taskén 2013). Therefore, it may be suggested that Tt.Cr can inhibit cyclooxygenase enzymes thus limiting the amount of prostaglandin available in hypothalamus and at nociceptors.

The witnessed spasmolytic outcomes of *T. triquetra* on gastrointestinal, respiratory and cardiovascular systems might be facilitated by blocking Ca^{+2} channels and possibly provides logical foundation to authenticate the traditional remedial usage of herb. The detected lipoxygenase inhibition could endorse its ancient use in pain and inflammation. In the current study, crude extract is used, which comprises numerous components. Thus, advanced research must be conducted to isolate principal bioactive constituents accountable for the activities mentioned earlier and establish the test compound's safety and toxicity.

References

Adedapo AA, Sofidiya MO, Masika PJ and Afolayan AJ 2008. Anti-inflammatory and analgesic activities of the aqueous extract of acacia Karroo stem bark in experimental animals. Basic Clin. Pharmacol. **103**(5): 397-400.

Ahmed N, Mahmood A, Mahmood A, Sadeghi Z and Farman M 2015. Ethnopharmacological importance of medicinal flora from the district of Vehari, Punjab province, Pakistan. J. Ethnopharmacol. **168**: 66-78, doi:https://doi.org/10.1016/j.jep.2015.02.048.

Ahmad S, Wariss HM, Alam K, Anjum S and Mukhtar M 2014. Ethnobotanical studies of plant resources of Cholistan desert, Pakistan. Int. J. Sci. Res. **3**: 1782-1788.

Akbar G and Khatoon S 2012. Floral Guide of Indus Ecoregion. Pakistan WWF-Pakistan.

Asongalem E, Foyet H, Ekobo S, Dimo T and Kamtchouing P 2004. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. J. Ethnopharmacol. **95**(1): 63-68.

Bolton T 1979. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. **59**(3): 606-718.

Chitra M and Nithyanandhi K 2007. Radical scavenging activity of Trianthema triquetra in male albino rats intoxicated with CCl_4. J. Environ. Biol. **28**(2): 283.

Council NR 2010. Guide for the care and use of laboratory animals, National Academies Press.

Farre A, Colombo M, Fort M and Gutierrez B 1991. Differential effects of various Ca^{2+} antagonists. Gen. Pharmacol. **22**(1): 177-181.

Geusens P, Emans PJ, de Jong JJ and van den Bergh J 2013. NSAIDs and fracture healing. Curr. Opin. Rheumatol. **25**(4): 524-531.

Ghori SSaH, T. 2016. Evaluation of ethanolic extract of roots of *Trianthema triquetra* for anti-ulcer activity in albino rats. World J. Pharm. Life Sci. **2**(5): 385-392.

Hill-Eubanks DC, Werner ME, Heppner TJ and Nelson MT 2011. Calcium signaling in smooth muscle. CSH Perspect. Biol.: a004549.

Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K-I, Harada K-I, Miyamoto S, Nakazawa H, Won K-J and Sato K 1997. Calcium movements, distribution, and functions in smooth muscle. Pharmacol. Rev. **49**(2): 157-230.

Katewa S and Galav P 2005. Traditional herbal medicines from Shekhawati region of Rajasthan.

Khan A, Rahman M and Islam S 2008. Antipyretic activity of *Peperomia pellucida* leaves in rabbit. Turk. J. Biol. **32**(1): 37-41.

Lone AM and Taskén K 2013. Proinflammatory and immunoregulatory roles of eicosanoids in T cells. Front. Immunol. **4**: 130.

Loux J, Depalma P and Yankell S 1972. Antipyretic testing of aspirin in rats. Toxicol. Appl. Pharm. **22**(4): 672-675.

Quattrocchi U 2012. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology (5 Volume Set); CRC Press. pp.

Sathyaraj K and Indumathy R 2018. Evaluation of in vitro anti-inflammatory activity of whole plant of *Trianthema triquetra* rottler ex wild. World J. Pharm. Sci. **7**(5): 1781-1790.

Schaible A, Koeberle A, Northoff H, Lawrenz B, Weinigel C, Barz D, Werz O and Pergola C 2013. High capacity for leukotriene biosynthesis in peripheral blood during pregnancy. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA) **89**(4): 245-255.

Saqib F, Jabeen N, Riaz M and Gabriela Sechel 2019. Evaluation of smooth muscle relaxant potential of *Bismarckia nobilis* (Hildebr. & Wendl.) in diarrhea, hypertension and asthma by *ex vivo* and *in vivo* method. Bull. Lat. Am. Carib. Plant. Med. Arom. **18**(2): 204 -222.

Saqib F, Mushtaq Z, Janbaz KH, Imran I, Deawnjee S, Haq MZU and Dima L 2018. Pharmacological basis for the medicinal use of *Michelia champaca* in gut, airways and cardiovascular disorders. Asian Pacif. J. Tropic. Med. **11**(4): 292-298.

Saqib F and Janbaz KH 2016. Rationalizing ethnopharmacological uses of *Alternanthera sessilis*: A folk medicinal plant of Pakistan to manage diarrhea and asthma. J. Ethnopharmacol. **182**: 110-121.

Saqib F**,** Ahmed MG, Janbaz KH, Dewanjee S, Jaafar HZE and Haq MZU 2015. Validation of ethnopharmacological uses of *Murraya paniculata* in disorders of diarrhea, asthma and hypertension. BMC Compl. Alt. Med. **15**: 319-326.

Tappel A 1953. The mechanism of the oxidation of unsaturated fatty acids catalyzed by hematin compounds. Arch. Biochem. Bioph. **44**(2): 378-395.

Tona L, Kambu K, Ngimbi N, Cimanga K and Vlietinck A 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol. **61**(1): 57-65.

Van-Rossum J 1963. Cumulative concentration-response curves. II. Techniques for the making of concentration-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther. **143**: 299-330.

Zereen A, Khan ZU and Sardar AA 2016. An assessment and documentation of conventional medico-botanical knowledge of indigenous communities around chichawatni plantation reserved forest. Pharm. Chem. J. **2**: 27-35.

(Manuscript received on 15 April, 2019; revised on 11 March, 2020)