Research Article

Antiasthmatic, Antispasmodic and Tocolytic Effect of 80% Methanolic Extracts of Leaves of *Viburnium Linn.* Species – A Comparative *In vitro* Evaluation

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ABSTRACT

The genus, *Viburnum* Linn., belonging to the family, Adoxaceae (formerly positioned under the family - Caprifoliaceae), has been surveyed to cover about 200 species, in the world, and about 17 of them in India, especially, at an altitude from 800 – 2500 ft, habitat such as Himalaya and Nilgiri hills. The leaf parts of these species claim to contain an appreciable quantity of therapeutically valuable phenolic compounds like anthocyanins, phenolic acids, flavones, flavonols and biflavone, and their glycosides. Based on the above facts on records, the present study has been under taken. With an objective of screening the antiasthmatic, antispasmodic and tocolytic potentials 80% v/v methanolic leaf fractions of some three species of this genus, namely: *V. punctatum*, *V. coriaceum* and *V. erubescens* by *in vitro* evaluation method. From the findings of percentage inhibition, it is concluded that extract of *V. erubescens* possessed a significant antiasthmatic, antispasmodic and tocolytic activities ($p<0.01$). However, the magnitude of the activities potential among the species was not far different. This study can be a referential tool for the *in vivo* studies and isolation of active constituents which are responsible for the above biological activity and to conduct an advanced scientific investigation on these species in that regard.

Keywords: *Viburnum*, *in vitro*, Tocolytic, antispasmodic, antiasthmatic, Adoxaceae.

INTRODUCTION

Viburnum Linn. Species contain sterols, sesqui and triterpenoids, phenolic compounds and their glycosides as their common chemical constituents[1-5]. A few species among 17 in India, namely: *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume and *Viburnum erubescens* Wall.ex DC; have been reported in literature to posses uterine sedative, anti-asthmatic, astringent, anti-inflammatory and anti-microbial activities[6,7]. A verbal enquiry to the local community and plant vendors of Ooty and Coimbatore, Tamilnadu, also supported that the above listed pharmacological activities were traditional and were promising with roots, stem barks and leaves of these species[8].

Among the above listed chemical constituents, phenolic compounds, terpenoids and their glycosides may be the cause for biological responses. In addition to this, a qualitative chemical screening was performed to reveal that the leaf part of these three species contains an appreciable amount and a wide range of phenolic compounds[9,10].

Radical scavenging activities of phenolic compounds play a key role in ameliorating healing and even preventing several ailments in living being. It is a well known fact that the plants synthesis phenolic compounds for diverse purposes, which may be of protective, functional or as metabolic end products in nature[11]. But, human exploit them as valuable medicines/ phyto-pharmaceuticals by focusing on their anti-oxidant potential with or without modification.

A quest for a search of herbal phenolic compounds is still a renewed interest in the science of natural products as a source of valuable medicines. The herbal phenolic

molecules such as flavonoids, anthocyanins, bioflavones and other phenolic glycosides have, already, been explored and known for their applications against several human ailments-cardiovascular disorders, chronic inflammation and GIT related troubles[12-14]. So, it was decided to experiment the methanolic leaf fractions of all three species to screen for antiulcer potentials using suitable experimental animals against appropriate standard drugs.

MATERIALS AND METHODS

Plant Material

The research specimens for the present study was collected from Nilgiri hills and taxonomically authenticated by Dr. Chelladurai, (Ex. Professor) Medicinal plants supply for siddha, Govt. of India, Tamilnadu as *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume. and *Viburnum erubescens* Wall.ex DC. Herbarium of the specimens (labelled V181, VC131 and VE131 respectively) was submitted to the museum of the department of Pharmacognosy and Phytochemistry, at Vivekananda Group of Institutions, Batasingaram, Telangana, India. The leaves were dried in the shade for a couple of weeks and separately ground in a mechanical grinder to obtain moderately coarse powder. About 1 kg of leaf powder of each species was soxhleted for 15 – 18 h successively with petroleum ether (40 - 60ºC), chloroform and 80 % v/v aqueous methanol followed by determination of percentage methanolic extractives. The methanolic leaf fractions of *V. punctatum*, *V. coriaceum* and *V. erubescens* were labelled to be VPMLF, VCMLF and VEMLF respectively, and the extracts were screened for their chemical fractions with aid of suitable reagents and methods and then subjected to biological screening.

Animals

The animals used throughout the study were housed under standard laboratory conditions in polyacrylic cages, and were provided with pelleted food and water *ad libitum*. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of Nova College of Pharmacy, Jangareddygudem, Andhra Pradesh, India, and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The statistical analysis of study was carried out using one way analysis of variance (ANOVA) followed by Dunnett's 't' test and all calculations were performed using Graph-pad Prism software, $p<0.01$ was accounted significant.

IN VITRO ANTIASTHMATIC ACTIVITY STUDIES

Isolated guinea pig ileum preparation[15]

Guinea pigs weighing (300-500 g), starved over night with water *ad libitum*. The animal was killed by a blow on the head and the neck was exsanguinated. The abdomen was cut open and a suitable length of the ileum (approximately 2 cm long) was placed on a petridish containing Tyrode solution. The composition of the Tyrode solution in mM was NaCl 137 mM, $NaHCO_3$ 12 mM, NaH_2PO_4 0.3 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.0 mM and d- glucose 5.6 mM. Experiment were performed in a 30 ml organ bath containing Tyrode solution maintained at 37^0 C under a tension of 0.5 gm and gassed with air mixture (O_2+CO_2). Isometric contractions were recorded in a smoked kymograph paper with frontal writing lever. After an equilibration period of 30 min during which the Tyrode solution was changed intervals of 10 minutes, contractile responses were recorded for histamine (10 µg/ml) and acetylcholine (10 µg/ml). The contact time of 30 sec recorded at 5 min time cycle is kept for proper recording of the responses. The extracts - tissue contact time was 1 min before the addition of histamine and acetylcholine. Thus, the effect of the extract on histamine and acetylcholine-induced contractions were recorded. The percentage inhibition of the extracts on contraction induced by histamine and acetylcholine was calculated.

Isolated rat fundus strip preparation [16]

Wistar rats weighing (150-200 g), were starved over night with water *ad libitum*. The animals were sacrificed by cervical decapitation. The abdomen was cut open the stomach was exposed. The fundus of the stomach was identified. Incised it from the junction of pyloric part and transferred it the petri dish containing Tyrode solution. Incise the fundus from the lesser curvature and open it longitudinally and was cut in to sheet, from where strips of about 2-3 cm long were prepared. The composition of the Tyrode solution in mM was NaCl 137 mM, $NaHCO_3$ 12 mM, NaH_2PO_4 0.3 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.0 mM and d-glucose 5.6 mM. The strip was suspended in atropinized Tyrode solution ($3.5x10^{-7}$M). The experiments were performed in a 30 ml organ bath containing Tyrode solution maintained at 37^0 C under a tension of 1.0 gm and gassed with air mixture (O_2+CO_2). Isometric contractions were recorded in a smoked drum

using a frontal writing lever. After an equilibration period of 60 min, contractile responses were recorded for 5-hydroxytryptamine (10 µg/ml). The contact time of 90 sec, and 5 min time cycles are kept for proper recording of the responses. Extracts- tissue contact time was 1 min before the addition of 5-hydroxytryptamine. The effects of the extract on 5-hydroxytryptamine induced contractions were recorded. The percentage inhibition of the extracts on contraction induced by 5-hydroxytryptamine was calculated.

IN VITRO ANTISPASMODIC AND TOCOLYTIC ACTIVITY STUDIES

Preparation of guinea pig ileum for anti-spasmodic activity[17]

Guinea pigs weighing (300-500 g), starved over night with water *ad libitum*. The animal was sacrificed by a blow on the head and the neck was exsanguinated. The abdomen was cut open, the mesentery was trimmed followed by removal of the content of the lumen and a suitable length of the ileum (approximately 2 cm long) was placed on a petridish containing Tyrode solution. The composition of the Tyrode solution in mM was NaCl 137 mM, $NaHCO_3$ 12 mM, NaH_2PO_4 0.3 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.0 mM and d-glucose 5.6 mM. the study was performed in a 30 ml organ bath containing Tyrode solution maintained at 37°C under a tension of 0.5 gm and aerated with air mixture (O_2+CO_2). Isometric contractions were recorded in a smoked kymograph paper with a frontal writing lever. After an equilibration period of 30 min during which the Tyrode solution was changed intervals of 10 minutes, contractile responses were recorded for histamine (10 µg/ml) and acetylcholine (10 µg/ml). The recordings were carried out keeping 5 min time cycles with 30 sec contact-time. The VCME, VEME and VPMEwere added about 1 min before the addition of histamine and acetylcholine. Thus, the effect of the extract on histamine and acetylcholine-induced contractions were recorded. The percentage inhibition by various concentrations of VCME, VEME and VPMEto the contractions induced by histamine and acetylcholine was calculated.

Tocolytic potential of VPMLF, VCMLF and VEMLF

Uterine muscle relaxant effect of methanolic extracts (10 mg/ml) of each VPMLF, VCMLF and VEMLF was screened on the isolated uterus preparation, which was dissected from a healthy female rat (2 cm tissue; De Jalon solution with 7.4 pH, at 29º C) against oxytocin induced rhythmic contractions. The tissue preparation was exposed to oxytocin (0.01 U/ml) and the contractile response was recorded. The test samples (10 mg/ml each VPMLF, VCMLF and VEMLF) then, were administered along with oxytocin to trace out spasmolytic effect of test samples.

The percentage reduction of amplitude of contractions with respect to the normal oxytocin contractions was calculated[18, 19].

RESULT AND DISCUSSION

Mediators like histamine, serotonin, and acetylcholine play major role in various ways in the pathogenesis of Asthma. Among all histamine predominantly mediates broncho constriction that accompany asthma. Serotonin in asthma is uncertain but a potent broncoconstrictor and also inverses acetylcholine release from airway nerves via $5HT_3$ receptor. Methanolic extracts of *Viburnum* Linn. species inhibited the histamine and acetylcholine induced contraction of guinea pig ileum significantly ($p<0.01$) and the inhibition may be attributed to its antihistaminic/anticholinergic activity. The antiasthmatic acticity was ascending in series *V. erubescens* < *V.coriaceum* < *V. punctatum.*

The complete inhibition on histamine induced contractions was achieved in 32 mg/ml, 16 mg/ml and 16 mg/ml with VPMLF, VCMLF and VEMLF respectively.

On rat fundus strip methanolic extracts exhibited mild inhibitory activity against 5-HT induced contraction. The 100% inhibition of 5-HT induced contractions was achieved by VPMLF, VCMLF and VEMLF in 128 mg/ml, 64 mg/ml and 32 mg/ml respectively.

A complete blockade of spasmogenic potential of acetylcholine was achieved at dose level of 32 mg/ml, 16 mg/ml and 16 mg/ml with VPMLF, VCMLF and VEMLF respectively. (Table 3.5.2).

The contractile pattern of test samples with oxytocin in combination was then compared with the spasmolytic effect of aspirin (1 µg/ml) with oxytocin (0.01 U/ml) in combination. Tocolytic potential of the test drugs was comparable and significant ($p<0.05$) to that of the aspirin-induced spasmolytic response ($p<0.01$)).

Table 1: Effect of VCME, VEME and VPME on Histamine induced contractions on isolated guinea pig ileum

S.No.	Drug Treatment		Mean contraction (mm)		
	Histamine (µg/ml)	Extract (mg/ml)	VPMLF	VCMLF	VEMLF
1.	8.0	-	56.05±6.17	52.63±4.06	55.70±3.31
2.	8.0	2	47.50±2.43 (14.02)*	44.81±2.13 (15.31)*	40.32±2.81 (23.55)*
3.	8.0	4	33.61±1.85 (38.20)*	20.87±3.01 (59.63)*	17.16±2.52 (69.37)*
4.	8.0	8	22.90±1.53 (59.14)*	17.44±1.82 (72.94)*	6.04±0.35 (89.45)*
5.	8.0	16	16.11±0.89 (73.37)*	0±0 (100)*	0±0 (100)*
6.	8.0	32	0±0 (100)*	-	
7.	8.0	64	-	-	

*N=4, Values are mean±SEM, *p<0.01 when compared with control (maximum response was taken as 100% response), values in parenthesis percentage inhibition*

Table 2: Effect methanolic extracts on 5-hydroxy tryptamine induced contractions of guinea pig ileum

S. No	Treatment		Mean contraction (mm)		
	5-hydroxy tryptamine (µg/ml)	Extract (mg/ml)	VPMLF	VCMLF	VEMLF
1	8.0	-	17.01±1.05	16.49±2.15	16.77±3.41
2	8.0	1	15.17±1.33 (11.96)*	14.67±1.73 (12.86)*	13.60±2.03 (16.42)*
3	8.0	2	14.55± 2.12 (17.54)*	12.46± 1.88 (21.34)*	12.09± 2.51 (25.36)*
4	8.0	4	13.40 ± 2.06 (23.45)*	10.03 ± 1.35 (37.65)	9.93 ± 2.04 (44.27)*
5	8.0	8	10.33 ± 0.86 (41.78)*	8.83 ± 1.60 (48.53)*	6.14 ± 0.97 (61.08)*
6	8.0	16	6.04± 0.21 (64.20)*	5.60± 0.43 (68.81)*	3.89± 0.19 (80.17)*
7	8.0	32	4.11 ± 0.27 (76.81)*	3.04 ± 0.21 (81.40)*	0 ± 0 (100)*
8	8.0	64	2.45 ± 0.51 (88.07)*	0 ± 0 (100)*	-
9	8.0	128	0 ± 0 (100)*	-	-

*Values are mean ±SEM of triplicate, *p<0.01 When compared with control (maximum response in mm was taken as 100% contraction), values in parenthesis percentage inhibition*

Table 3: Effect of VCMLF, VEMLF and VPMLF on acetylcholine induced contractions on guinea pig ileum

S. No.	Drug Treatment		Mean contraction (mm)		
	Acetyl choline (µg/ml)	Extract (mg/ml)	VPMLF	VCMLF	VEMLF
1.	8.0	-	41.13±3.67	37.03±1.82	40.56±4.03
2.	8.0	2	31.27±1.73*	27.08±3.04*	30.91±2.16*
3.	8.0	4	24.34±2.60*	19.67 ±1.21*	21.76±1.44*
4.	8.0	8	18.13±2.08*	11.08±0.34*	9.01±0.73*
5.	8.0	16	9.71±0.37*	2.93±0.08*	2.01±0.03*
6.	8.0	32	1.82±0.44*	0±0	0±0
7.	8.0	64	0±0	-	-
8.	8.0	128	-	-	-

*N=4, Values are mean±SD, *p<0.01 when compared with control (maximum response was taken as 100% response)*

Table 4: Effect of VCMLF, VEMLF and VPMLF, and aspirin on oxytocin induced contractions of isolated rat uterus

Groups	Drug Treatment	Response in mm				
		0min	30sec	60sec	90sec	120sec
I	Oxytocin (0.01U/ml)	19.12± 2.07	33.15± 3.76	43.06± 3.44	60.13± 5.01	74.04± 3.16
II	VPMLF(10µg/ml)+ Oxytocin (0.01U/ml)	11.08± 1.03*	17.20± 1.62*	26.73± 3.05*	37.14± 2.97*	45.90± 3.05*
III	VCMLF(10µg/ml)+ Oxytocin (0.01U/ml)	14.12± 1.42*	19.16± 1.16*	31.44± 2.50*	37.06± 2.83*	39.92± 3.10*
IV	VEMLF(10µg/ml)+ Oxytocin (0.01U/ml)	12.05± 1.03*	16.96± 1.43*	22.13± 1.63*	31.03± 2.09*	37.01± 1.43*
V	Aspirin (1µg/ml)+ Oxytocin (0.01U/ml)	5.86± 0.09**	10.11± 1.30**	15.00± 1.57**	21.45± 2.03**	28.14± 1.41**

*N=4, values are mean±SEM, 30 sec contact time, time cycle 3 min, *p<0.05, **p<0.01*

CONCLUSION

From the above observation it has been revealed that the successive 80% methanolic leaf fractions of *Viburnum* Linn. species exhibited an appreciable antiasthmatic (antagonistic tic to H_1 and M_3 receptors, and 5-HT receptors) and uterine relaxant (antagonistic to PGs or PGs based uterine contraction) activities which are comparable to the potential of a conventional NSAID. The findings of the current study will, surely, be useful to begin with *in vivo* pharmacological experimentation on these species to record their pharmacological values.

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