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Full Length Research Paper

Blood pressure lowering effect of *Tylophora hirsuta* wall

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Crude hydromethanolic extract of *Tylophora hirsuta* (Th.Cr) was studied in spontaneous hypertensive Wistar rats for possible effects on high blood pressure and heart rate. In the absence of atropine, fall in arterial blood pressure was 64±7 mmHg at the dose of 100 mg/kg while in the presence of atropine, there was no effect on arterial blood pressure suggesting cholinergic receptor blockade. Fall in heart rate, in the absence of atropine, was 218±8 beats per minute (BPM) while, fall in heart rate, in the presence of atropine was 110±5.6 BPM, indicating that the action is through cholinergic muscarinic receptors as atropine is a typical anticholinergic drug. The percent fall in blood pressure and heart rate were standardized versus acetylcholine; standard cholinergic drug. Th.Cr tested mildly positive for alkaloids, flavonoids and strongly positive for terpenes and terpenoids, and saponins. α- Amyrin acetate was isolated from the *n*-hexane fraction of Th.Cr for the first time from *T. hirsuta*. The results confirm the presence of acetylcholine like substances in *T. hirsuta* that provide a basis for its traditional use in treatment of hypertension. The action may be attributed to the phytochemicals like flavonoids, saponins, terpenes and terpenoids and α- amyrin acetate isolated for the first time from the plant.

Key words: *Tylophora hirsuta,* arterial blood pressure, antihypertensive, α- amyrin acetate.

INTRODUCTION

Tylophora hirsuta (common name: Tylophora) is a herb that belongs to family asclepiadaceae (Bashir et al., 2009a). Family asclepiadaceae is distributed mainly in the tropical and subtropical regions of the world with 175 to 180 genera and 2200 species as described in 'Flora of Pakistan' by Yasin (1983). While going through the literature, the family asclepiadaceae has great ethnobotanical importance in the treatment of different diseases (Kumar and Nagyan, 2006). For example, *Ceropegia bulbosa* is used in the treatment of digestive disorders and as tonic. Similarly, *Oxystelma racamone* is used in the treatment of sore throat and *Pentatropis spiralis* is used as emetic, astringent and in the treatment of gonorrhea. *Pergularia daemia* is used in the treatment of asthma, diarrhea, amenorrhea and other gastro-intestinal disorders. *Dregea volubilis* is used in the treatment of snake bite, skin infections like boils and cellulites. In Pakistan, the genus asclepiadaceae is represented mainly by two species that is, *T. hirsuta* and *T. tenerrima. Tylophora* is of great medicinal importance as it is used, traditionally, for the treatment of asthma, high blood pressure, diarrhea, rheumatism and other allergic conditions (Bashir et al., 2009b). Tylophora was listed as herbal medicines in the Bengal Pharmacopoeia since 1884. Other reported activities are anti-allergic and anti-arthritic activities (Gopalakrishnan et al., 1980; Wagner, 1989). Similarly, cyto-toxicity of *T. asthmatica* has been established in male rats (Dikshith et al., 1990). Previously, reported alkaloids from *T. hirsuta* are tylohersutinine, 13 a-methylo-hirsutine, 13a-methyltylo-hirsutinidine, tylohirsutinidine (Bhutani et al., 1984, 1985)

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Abbreviations: BPM, Beats per minute; **Th.Cr,** crude hydromethanolic extract of *Tylophora hirsuta*; **EtOAc,** ethyl acetate; **BuOH,** butanol; **$CHCl_3$,** chloroform.

with antiamoebic activity (Bhutani et al., 1987). Other constituents reported from the aerial parts of the *T. hirsuta* are Gymnorhizol, and β-sitosterol, a terpene (Ali and Bhutani, 1991). Keeping in view, traditional use of *T. hirsuta* in the management of high blood pressure, we designed our this study to screen the crude hydromel-thanolic extract of *Tylophora hirsute* (Th.Cr) for possible blood pressure lowering effect (Gilani and Aftab, 1992).

MATERIALS AND METHODS

Plant collection

The aerial parts of *T. hirsuta* were collected in April to May, 2005 from the nearby hills of University of Malakand. The plant was identified by Professor Dr. Jehandar Shah, plant taxonomist and ex Vice Chancellor, University of Malakand. A voucher specimen (Th. 0l- 2005) has been submitted to the herbarium of the University of Malakand.

Extraction and fractionation of extract

The shade dried materials (7.5 kg) were ground to fine powder. The powdered materials were macerated with hydromethanol (80%) for 15 days. The mixture was subjected to occasional shaking on alternate day. The materials were filtered with ordinary filter paper and the process was repeated thrice. All the filtrates were combined and concentrated under reduced temperature and pressure using a rotary evaporator. A brownish black extract (1100 g) was obtained. 1000 g of Th.Cr was suspended in sufficient distilled water and was successively fractionated with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), butanol (BuOH) and water that yielded, respectively, *n*- hexane (50%), $CHCl_3$ (4.5%), EtOAc (2.0%), BuOH (0.5%) and aqueous (25.0%) fractions.

Drugs and animals

Acetylcholine was purchased from BDH chemicals, Poole England. Atropine and other chemicals were purchased from Merck, Germany. Solutions and final dilutions were prepared on the same day of the experiments in double distilled water, sterilized by membrane filtration method. Spontaneous hypertensive Wistar rats (150-200 g) of either sex were used in the experiments. The animals were housed at the “Animal House of University of Malakand” complying with standards mentioned in the “Animals Bye-Laws, 2008 of the University of Malakand (Scientific procedures Issue- 1), under number UOM/Ethics/PharmaLab/ 01/2008. Standard diet and tap water were given to the animals.

Preliminary phytochemical screening and isolation of compound

Preliminary phytochemical screening of the Th.Cr was carried out for the presence of various phytochemical groups like alkaloids, flavonoids, terpenes, saponins and tannins (Aduragbenro et al., 2009; Harborne, 1973). Activity guided isolation was performed for isolation of pharmacologically active compound using column chromatography. Activity guided fractionation revealed that the pharmacologically active substance were in the *n*-hexane and $CHCl_3$ fraction (data not shown). Solvent system was developed using EtOAc / *n*-hexane mixture with increasing order of polarity. At a concentration of 7% EtOAc in n-hexane solvent system, clear bands of 3-4 compounds were observed. All the fractions were screened for possible antihypertensive activity. Th.Cr, *n*-hexane and $CHCl_3$ fraction gave antihypertensive activity. The *n*-hexane and $CHCl_3$ fraction was loaded in a column and subjected to column chromatography using *n*-hexane / EtOAc solvent system in increase order of polarity; 100% *n*-hexane, 2% EtOAc, 4% EtOAc, 6% EtOAc till 100% EtOAc was passed through the column.

Major compound 1 (6.5 g) was isolated at 3.5% EtOAc / *n*-hexane solvent system in a separate pencil column. Spectroscopic techniques like HREIMS, FAB positive and FAB negative, IR, UV, ^{13}C-NMR, ^{1}H-NMR, and optical activity were used to determine the structure of the compound. Saturated ceric sulfate solution in 65% sulphuric acid was used as spraying reagent to visualize the compounds.

Statistical analysis and interpretation of data

Chart 5 for Windows, purchased from AD Instruments Australia was used to interpret the graph tracings. Heart rate was calculated by using software in channel 2 with input from channel 1 of the software. Student “t” test was used at 95% confidence interval (CI). ‘P’ values less or equal to 0.05 was considered as statistically significant.

Cardiovascular effects in anesthetized rats

Invasive model was used as it gives more precise results than non invasive model that requires control experimental parameters like body temperature (27°C), respiratory and body movements, and noise (Aziz et al., 2009). Spontaneous hypertensive Wistar rats (150-200 g) of either sex were anaesthetized with thiopental sodium at a dose of 80.0 mg/kg (i.p.). Their tracheas were opened and canulated to facilitate spontaneous respiration. The external jugular vein was canulated for administration of drugs. Similarly, the carotid artery was canulated and attached to Bridge Amplifier ML221 connected with Power lab (Model No: 4/25 T) to record the arterial pressure and heart rate. The canulated artery was maintained open by a small flush of heparinized solution (40 IU/ml) prepared in normal saline to prevent the coagulation of blood in the canulated artery. Earlier, the animals were equilibrated for at least 30 to 35 min before the administration of drug. A soft tissue paper soaked in normal saline was placed on the exposed incised parts of test animal to maintain it moist. Moreover, the entire process was performed under ordinary table lamp at suitable distance that also helped keeping the test animals warm. The standard solutions of nor epinephrine (1.0 µg/kg), acetylcholine (1.0 µg/kg) and test samples were administered through the jugular vein cannula with a small flush of normal saline so that complete test dose reached the circulation. Th.Cr at test doses of 1, 3, 10, 30, 100 and 300 mg/kg were slowly injected into the jugular vein as per reported procedure (Gilani and Aftab, 1992). According to the procedure, duration of 1 min was enough to compare the result of test with standard drug. The changes in the arterial blood pressure and heart rate beat per minute (BPM) were recorded. Blood pressure and heart rate before experimentation of test doses were taken as control. In order to explain its possible mode of action, in another series of experiments, we administered atropine at a dose of 1 mg/kg. The test samples were tried in similar way as described above. The treatment of atropine blocked the fall in blood pressure. The results were noted.

RESULTS AND DISCUSSION

Th.Cr tested mildly positive for the presence of alkaloids

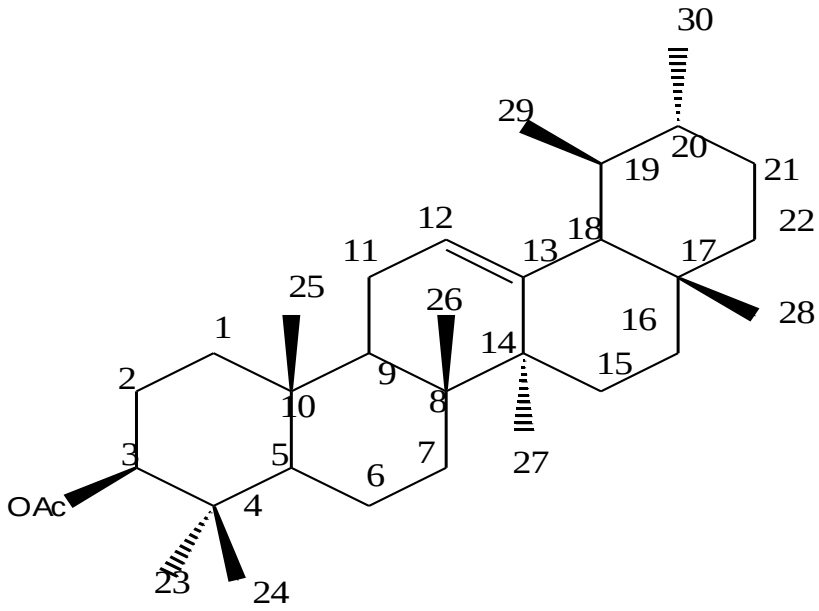

Figure 1. Structure of α-amyrin acetate isolated from *T. hirsuta* wall.

and flavonoids. However, it tested strongly positive for saponins, terpenes and terpenoids. It gave negative tests for tannins. Our interest was in the isolation of terpenes and activity guided isolation of pure compound as it tested strongly positive. The *n*-hexane and $CHCl_3$ fractions were pharmacologically active giving strongly positive tests for the presence of terpenes. Activity guided isolation through column chromatography on flesh silica gel afforded major compound (α- amyrin acetate) that have been previously reported to have antihypertensive activity (Figure 1) using same invasive techniques for determining its effects on arterial blood pressure and heart rate (Rehana et al., 2001). Thus, the antihypertensive effect of the Th.Cr may be due to α-amyrin acetate, a triterpene, and other phytochemical constituents such as saponins, flavonoids and triterpenes present in the plant. On the experimentations, results of different test doses of Th.Cr are summarized in Table 1, showing dose dependent fall in the arterial pressure and heart rate of the rats. There is no statistically significant effect on the arterial pressure and heart rate with test doses of 1 and 3 mg/kg. Significant effects (95% confidence interval) on mean arterial blood pressure (in non atropinized animals) were observed in doses of 10.0, 30.0, 100.0 and 300.0 mg/kg with their corresponding values (mmHg ± SEM) 23 ± 3, 66 ± 6.9, 64 ± 7 and 113 ± 9 . Because of the median lethal dose LD_{50} = 650 mg/kg (unpublished data of the author), test dosing 10.0, 30.0, 100.0 and 300.0 mg/kg of reported procedure was adopted. Similarly, (in non atropinized animals) fall in the heart BPM was prominent with the dose of 100.0 mg/kg and extremely severe (fatal bradycardia) with 300 mg/kg. It is noteworthy that the drug reduced the blood pressure and produced bradycardia with higher doses. The results suggest that the plant have antihypertensive activity in small doses. However, effects on the heart were prominent with large doses.

The duration of the fall in blood pressure lasted for 1 min in the doses of 10 and 30 mg/kg like that of acetylcholine, a standard cholinergic drug. Blood pressure lowering effect at a dose of 300 mg/kg lasted for 3 to 4 min, followed by fatal bradycardia that was reversed by administration of 1.0 µg/kg solution of noradrenalin (data not shown). The reversal by epinephrine indicated that the effects were surmountable and mediated probably through muscarinic cholinergic receptors (Gilani and Aftab, 1992). The fall in blood pressure and heart rate were quantified as percent acetylcholine (1.0 µg/kg) in test animals (Figure 2). Fall in mean arterial blood pressure was 37.5, 75.1, 104.5 and 184.58% of the acetylcholine observed with respective test doses of 10.0, 30.0, 100.0 and 300.0 mg/kg of Th.Cr. Similarly, fall in the heart rate at the said test doses was 15.4, 27.7, 92.5 and 672% of acetylcholine. In atropine pretreated animals, the fall in blood pressure was blocked (Table 1) indicating that the falls in blood pressure and heart beats are due to the muscarinic receptors present in the blood vessels and the heart (Arunlakhshana and Schild, 1959; Furchgott and Zawadzki, 1990; Gilman et al., 1990). However, at dose 300.0 mg/kg (in atropine pretreated animals), atropine was probably dislodged from cholinergic muscarinic receptors and consequently a relatively low fall in heart rate was observed. This partial blockade by the higher doses of extract (300.0 mg/kg) further confirms the competitive antagonism phenomenon of the cholinergic muscarinic receptors (Gilani and Aftab, 1992). This work also, strongly endorses our previously reported work that the Th.Cr has cholinomimetic activity

Table 1. Effects of different doses of Th.Cr on arterial blood pressure and heart rate of anesthetized rats in the presence and absence of atropine.

Control*		Dose (mg/kg)	Fall in		Calculated t value †	Fall in		Calculated t value §
Mean arterial blood pressure † (mm Hg ± SEM)	Mean heart rate § (BPM± SEM)		Mean arterial blood pressure † (mm Hg ± SEM) without atropine	Mean arterial blood pressure (mm Hg ± SEM) with atropine		Heart rate § (beats/min± SEM) without atropine	Heart rate (beats/min± SEM) with atropine	
		1	0	0	0	0	0	0
		3	0	0	0	0	0	0
170 ± 5	370 ± 6.5	10	23 ± 3	0	3.21	5 ± 0.8	0	0.76
		30	46 ± 6.9	0	5.39	9 ± 2	0	1.32
		100	64 ± 7	0	7.43	30 ± 4.5	0	3.79
		300	113 ± 9	34 ± 4	10.97	218 ± 8	110± 5.6	21.14

All values are mean ± standard error of the mean (SEM); n=6. *, Control values were obtained immediately before the administration of test substance; †, link for calculated t value regarding changes in mean arterial blood pressure (level of significance; $p \leq 0.05$); §, link for calculated t value regarding changes in heart rate (level of significance; $p \leq 0.05$).

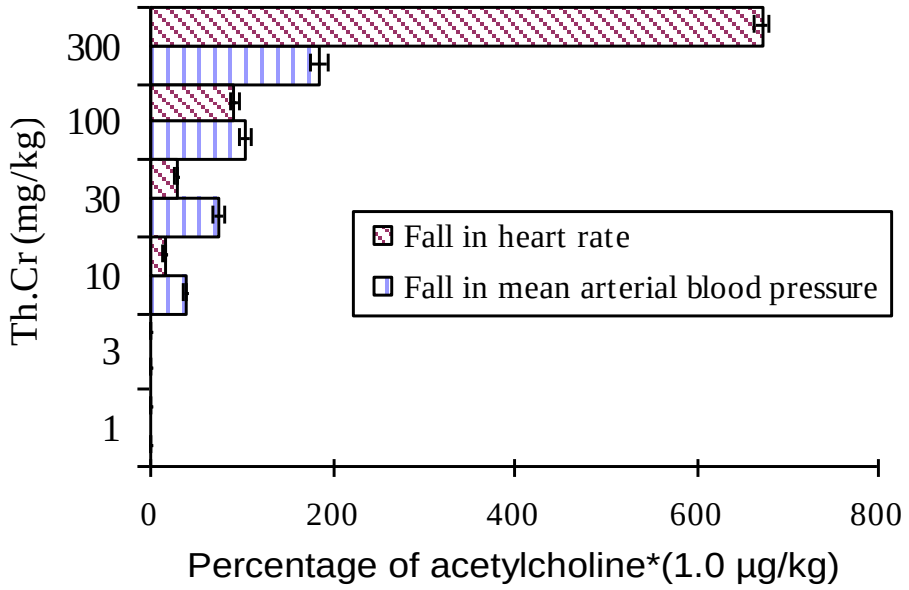

Figure 2. Effects of Th.Cr to show comparative fall in mean arterial blood pressure and heart rate in anaesthetized Wistar rats. All values are mean ± SEM; n=6. *Acetylcholine was used as standard cholinergic drug ($p \leq 0.05$).

(Bashir et al., 2009a).

Conclusion

The results indicate the presence of acetylcholine like substances and α-amyrin acetate in *T. hirsuta* that are responsible for fall in blood pressure and heart rate and thus, confirm traditional use of Tylophora in the treatment of hypertension.

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