# Interaction of Folk Medicinal Plant Extracts with Human $\alpha_2$-Adrenoceptor Subtypes

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Forty-two extracts of folk medicinal plant organs from Pakistan were tested in competition binding assays for their interaction with the specific ligand recognition sites on the human $\alpha_2$-adrenoceptor subtypes $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$. Strong binding of the extracts (40 mg/ml) from *Acacia nilotica* (L.) Delile leaves (88–98% displacement of radiolabel) and *Peganum harmala* seeds (89–96% displacement) on three subtypes prompted us to extract these plant materials with 40% and 80% methanol, ethanol, and acetone. The extraction results indicated an absence of $\alpha_2$-adrenoceptor binding activity in the stalk of *A. nilotica* and *A. tortils*, whereas the leaves of both plants contained activity. The extracts of *A. nilotica* leaves showed a slight, but consistent, preference for the $\alpha_{2C}$-adrenoceptor, whereas the leaves of *A. tortils* were slightly more active on the $\alpha_{2B}$ subtype. The extract of *P. harmala* stalks was less active than that of its seeds. The binding activities of *A. nilotica* leaves and *P. harmala* seeds were mainly concentrated in the water and 30% methanol fractions and further sub-fractions. In a functional activity assay, the active fractions inhibited epinephrine-stimulated $^{35}$S-GTP$\gamma$S binding, thus indicating a predominantly antagonistic nature of the compounds with $\alpha_2$-adrenoceptor affinity in these fractions. Among the known major alkaloids of *P. harmala* (demissidine, harmaline, harmine, 6-methoxyharmalan, and norharmane), only 6-methoxyharmalan showed moderate affinity (dissociation constant ($K_i$) of 530 ± 40 nM for $\alpha_{2A}$ subtype). This study is a first systematic attempt towards the discovery of potential drug candidates from these plant materials for treating $\alpha_2$-adrenoceptor related diseases.

## Introduction

The population of developing countries relies to about 80% on traditional medicines for their primary health needs and 85% of these traditional medicines involve the use of plant extracts. This means that about four billion people depend on natural products as their primary source of medication (Fransworth, 1988). However, natural products do not only play an important role in the formulation of traditional medicines in developing countries, they also have contributed in a major way to the discovery of drugs in developed countries. Despite the fact that combinatorial chemistry has dramatically increased the number of test compounds available for drug discovery, it is estimated that about half of the best selling drugs, and many drug candidates under development, are based on natural products (Mueller, 1999).

In Pakistan, a huge variety of medicinal plants are available (Dastur, 1970). Most of these plants are being used for therapeutic purposes without specific knowledge of their active ingredients. In fact, Pakistani medicinal plants, for the purpose of drug development, are one of the least investigated sources of natural compounds (Satyavati *et al.,* 1976). Even when these plants have been examined for their potential therapeutic usefulness, their possible interaction with cell surface receptors has been investigated very little. One of the few exceptions is a study by Rommelspacher *et al.* (1987), which showed harmaline (an indole alkaloid of *P. harmala* seeds) to possess agonistic activity on unclassified $\alpha_2$-receptor subtypes in human and rat tissues.

As part of a wider systematic study of much neglected Pakistani medicinal plants, extracts of forty-two plant organs, belonging to 21 botanical families, were tested for their binding affinities on

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human $\alpha_2$-adrenoceptor subtypes, which represent important therapeutic targets for the treatment of CNS-related diseases (Kobilka, 1999). They have a marked impact on human physiology, pathology and psychology and are among the best characterized members of the large super-family of G-protein-coupled receptors mediating the response of cells to hormones and neurotransmitters (Ruffolo *et al.,* 1993). In order to estimate their binding affinity on human $\alpha_2$-adrenoceptors, the extracts were tested in radioligand competition assays on $\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$ adrenoceptor subtypes. In the first screening, the extracts of *P. harmala* seeds and *A. nilotica* leaves, showed significant activity towards $\alpha_2$-adrenoceptors. The crude extracts of these plant organs were therefore subjected to chromatographic fractionation guided by receptor-binding activities.

## Materials and Methods

### *Plant collection, identification and documentation*

The plants (Nasir and Ali, 1972) were collected from their habitats in various regions of Pakistan during March-September, 1997 and were identified by Professor Zahoor Ahmed, Department of Taxonomy, University of The Punjab, Lahore, Pakistan. Specimens were deposited in the Turku University Herbarium (TUR) (Saleem *et al.,* 2001).

### *Preparation of crude extracts*

Dried plant organs (500 mg) were homogenized for 40 sec with an Ultra-Turrax T25 (Janke and Kunkel, IKA-Labortechnik, Germany) with 40% or 80% solvent (methanol, ethanol or acetone) at room temperature. The homogenates were continuously stirred for 1 h and centrifuged at 1500 × *g* for 10 min. The pellets were extracted thrice and the extracts evaporated to dryness under reduced pressure at 30 °C. Dried extracts were re-dissolved in de-ionized water at 40 mg/ml and stored at – 18 °C until needed.

### *Extraction and alpha2-adrenergic receptor binding activity guided and fractionation*

Dried leaves of *A. nilotica* (carefully separated from the stalks) and seeds of *P. harmala* (200 g each) were extracted (4 ×) with 1 l of 80% acetone by continuously stirring for 1 h. The resulting pellets were combined and evaporated to dryness and re-dissolved in 50 ml de-ionized water and centrifuged at 3000 × *g* for 15 min and vacuum filtered through Buechner funnel containing round filter paper (110 mm) (Schleicher & Schuell, Dassel, Germany) into a 500 ml Erlenmeyer filtering flask. The resulting extracts were fractionated with size exclusion chromatography using Sephadex LH-20 column (40 × 3.8 cm i.d., Pharmacia, Umeå, Sweden) by consecutive elution with 150 ml water, methanol (30%, 50%, and 80%), and acetone (20%, 40%, 60%, and 80%). The resulting fractions were checked for their pharmacological activity and the active fractions were re-eluted on Sephadex LH-20 column with water, 5%, 10%, 15%, 20%, 25%, 30%, 50%, 80%, and 100% methanol. The water (Ph1, and An1) and 30% methanol (Ph2 and An2) fractions were re-eluted on Sephadex LH-20 column and 12 sub-fractions (50 ml each) of Ph1 and 8 sub-fractions of Phf2 (50 ml each) were collected by eluting with water and 5%, 10%, 15%, 20%, 25%, 30% and 50% methanol. An1 was eluted with water to collect four sub-fractions and An2 was eluted with water and 5%, 10%, 15%, 20%, 25%, and 30% methanol to collect eight sub-fractions. All fractions were adjusted at 40 mg/ml for further dilutions by re-dissolving in water and tested for their binding activities.

### *Pharmacological testing*

The testing for affinity on the three human $\alpha_2$-adrenoceptor subtypes was carried out in the form of competition binding with [$^3$H]-rauwolscine as the radioligand. The biological material consisted of membranes (13–15 μg protein per sample) from Shionogi 115 (S115) mouse mammary tumor cells stably expressing either of the human $\alpha_2$-adrenoceptor subtypes $\alpha_{2A}$ (S115-C10), $\alpha_{2B}$ (S115-C2) or $\alpha_{2C}$ (S115-C4) (Marjamäki *et al.,* 1992). Membranes and 1 – 2 nM [$^3$H]-rauwolscine were incubated in 50 mM $KH_2PO_4$, pH 7.5 with single concentration (Fig. 1 and Table I) or at six concentrations (Fig. 1 and Tables I–IV) of the extracts and fractions. Each sample was run in duplicate. After 30 min at room temperature, the incubations were terminated by rapid vacuum filtration through glass-fiber filter. Filters were washed three

times with 5.0 ml ice-cold incubation buffer, dried, and counted for radioactivity in a scintillation counter (Wallac, Turku, Finland). The data were normalized against the results of control samples containing no competing ligand (100% specific binding) or a saturating concentration (100 μM oxymetazoline) of a competing ligand (0% specific binding). For experiments, in which dilution series of extracts/fractions were tested, data analysis was carried out by nonlinear least square curve fitting to four-parametric competition curves in order to determine $IC_{50}$'s, which were then converted to apparent Ki's by means of the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

The antagonist activity of active fractions was determined as their ability to competitively inhibit epinephrine-stimulated $^{35}$S-GTPγS binding to G-proteins (Jasper *et al.,* 1998) in the membranes of Chinese Hamster Ovary (CHO) cells stably transfected with either of the three human $\alpha_2$ subtypes (Pohjanoksa *et al.,* 1997). Membranes (2–6 μg of protein per sample) and six serial dilutions of extracts/fractions were pre-incubated for 30 min in 50 mM tris(hydroxymethyl)aminomethane 5 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 10 μM GDP, 30 μM ascorbic acid, pH 7.4 at room temperature. After pre-incubation, a fixed concentration of epinephrine (5 μM for $\alpha_{2A}$, 15 μM for $\alpha_{2B}$ and 5 μM for $\alpha_{2C)}$ and trace amounts of $^{35}$S-GTPγS (0.08 nM- 0.20 nM, specific activity 46.3 TBq/mmol) were added to the incubation mixture. After an additional 60 min at room temperature, the incubations were terminated by rapid vacuum filtration through glass fiber filter. Filters were washed three times with 5 ml ice-cold wash buffer (20 mM tris(-hydroxymethyl)aminomethane, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4 at room temperature), dried and counted for radioactivity in a scintillation counter.

## Results and Discussion

### *Screening of 42 extracts*

A study of 42 extracts of folk medicinal plant organs of Pakistani origin was undertaken to determine their binding potentials at the active sites of $\alpha_2$-adrenoceptor subtypes ($\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$). In the initial screening, the extracts of *Acacia nilotica* (L.) Delile (Mimosoideae) leaves + stalks and *Peganum harmala* L. (Zygophyllaceae) seeds + stalks showed strong (above 85%) displacement of specific radiolabel binding, while those of *Cassia fistula* L. (Caesalpinioideae) beans and *Nelumbium speciosum* seed-coat showed moderate (≥ 37%) displacements. However, the extracts of *Cassia fistula* leaves and flowers, *A. nilotica* beans (more common as a folk medicine as compared to leaves), and *P. harmala* leaves, showed negligible (below 15%) displacement of specific binding on the three receptors. Based on these screening results, *P. harmala* seeds seeds + stalks and *A. nilotica* leaves + stalks were chosen for further studies.

### *Affinity – guided extraction with six solvents*

In order to select a suitable solvent for large-scale extractions for preparative chromatography, *P. harmala* seeds and stalks, *A. nilotica* and *A. tortilis* leaves and stalks were extracted in six solvent systems comprising of 40% and 80% (methanol, ethanol, and acetone). The extraction results indicated overall very similar trends of activity in all six solvent systems and hence the exclusive choice of a particular solvent for large-scale extraction remained indecisive (Table I). This might be due to similar solubility characteristics of the biologically active compounds present in these plant materials in the applied extraction solvents.

In *P. harmala*, for further checking whether seeds or their stalks contain activity, both were carefully separated and extracted. The extracts of *P. harmala* stalks showed weaker ($K_i$ = 160–800 μg/ml) activity than the seeds ($K_i$ = 16–76 μg/ml). These results clearly indicate that the $\alpha_2$-adrenoceptor binding activity of *P. harmala* was mainly located in seeds extracts. The stalks and leaves of *A. nilotica* and *A. tortilis* were also separately extracted to determine which part contained the $\alpha_2$-adrenoceptor binding activity. The *A. nilotica* and *A. tortilis* stalks, extracted with six solvents, showed no activity, while their leaf extracts were strongly active when extracted with all solvents. The leaves of *A. nilotica* exhibited weaker but consistent preference for $\alpha_{2C}$-subtype while the leaves of *A. tortilis* showed a similar but slightly lower preference for the $\alpha_{2B}$-subtype. This may be attributable to different molecules responsible for the activity present in these plant materials. The affinity trend in *A. nilotica* leaves was $\alpha_{2C} > \alpha_{2A} > \alpha_{2B}$ while in *A. tortilis* leaves it was $\alpha_{2B} > \alpha_{2A} > \alpha_{2C}$ (Table I).

Table I. $K_i$ values (μg/ml) of plant organs after extraction with methanol, ethanol and acetone. The data is based on competition experiments against [$^3$H]-rauwolscine with six concentrations of extracts.

| Nomenclature | Part extracted | Extraction solvent | $K_i$ [μg/ml][a] | | |
|---|---|---|---|---|---|
| | | | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ |
| *Peganum harmala* L. | seeds | methanol 40% | 40 | 25 | 57 |
| *Peganum harmala* L. | seeds | methanol 80% | 24 | 16 | 43 |
| *Peganum harmala* L. | seeds | ethanol 40% | 23 | 37 | 59 |
| *Peganum harmala* L. | seeds | ethanol 80% | 49 | 45 | 76 |
| *Peganum harmala* L. | seeds | acetone 40% | 52 | 53 | 69 |
| *Peganum harmala* L. | seeds | acetone 80% | 16 | 24 | 57 |
| *Peganum harmala* L. | stalks | methanol 40% | 296 | 229 | 667 |
| *Peganum harmala* L. | stalks | methanol 80% | 242 | 229 | 421 |
| *Peganum harmala* L. | stalks | ethanol 40% | 276 | 229 | 667 |
| *Peganum harmala* L. | stalks | ethanol 80% | 200 | 200 | 741 |
| *Peganum harmala* L. | stalks | acetone 40% | 160 | 186 | 727 |
| *Peganum harmala* L. | stalks | acetone 80% | 267 | 174 | 800 |
| *Acacia nilotica* (L.) Delile. | leaves | methanol 40% | 76 | 104 | 53 |
| *Acacia nilotica* (L.) Delile. | leaves | methanol 80% | 49 | 91 | 41 |
| *Acacia nilotica* (L.) Delile. | leaves | ethanol 40% | 68 | 110 | 25 |
| *Acacia nilotica* (L.) Delile. | leaves | ethanol 80% | 53 | 105 | 47 |
| *Acacia nilotica* (L.) Delile. | leaves | acetone 40% | 56 | 121 | 36 |
| *Acacia nilotica* (L.) Delile. | leaves | acetone 80% | 49 | 105 | 45 |
| *Acacia nilotica* (L.) Delile. | stalks | methanol 40% | n.a. | n.a. | n.a. |
| *Acacia nilotica* (L.) Delile. | stalks | methanol 80% | n.a. | n.a. | n.a. |
| Acacia nilotica (L.) Delile. | stalks | ethanol 40% | n.a. | n.a. | n.a. |
| *Acacia nilotica* (L.) Delile. | stalks | ethanol 80% | n.a. | n.a. | n.a. |
| *Acacia nilotica* (L.) Delile. | stalks | acetone 40% | n.a. | n.a. | n.a. |
| *Acacia nilotica* (L.) Delile. | stalks | acetone 80% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | methanol 40% | 36 | 25 | 46 |
| *Acacia tortilis* (Forsk.) Hyne | leaves | methanol 80% | 49 | 29 | 65 |
| *Acacia tortilis* (Forsk.) Hyne | leaves | ethanol 40% | 67 | 24 | 63 |
| *Acacia tortilis* (Forsk.) Hyne | leaves | ethanol 80% | 61 | 31 | 83 |
| *Acacia tortilis* (Forsk.) Hyne | leaves | acetone 40% | 41 | 31 | 63 |
| *Acacia tortilis* (Forsk.) Hyne | leaves | acetone 80% | 59 | 29 | 64 |
| *Acacia tortilis* (Forsk.) Hyne | stalks | methanol 40% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | methanol 80% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | ethanol 40% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | ethanol 80% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | acetone 40% | n.a. | n.a. | n.a. |
| *Acacia tortilis* (Forsk.) Hyne | stalks | acetone 80% | n.a. | n.a. | n.a. |

n.a. = no activity; [a] extracts with $K_i$ values higher than 800 μg/ml were considered inactive.

*Affinity-guided fractionation of extracts of P. harmala seeds and A. nilotica leaves*

The strong binding results of *P. harmala* seeds and *A. nilotica* leaves encouraged us to subject their crude extracts to adrenergic receptor binding activity guided fractionation. The 80% methanol extracts of each plant organ were separated by size exclusion chromatography using Sephadex LH-20 column. Each fraction (40 mg/ml) was checked for its $K_i$ and $K_B$ values after further dilutions at μg/ml level. In *A. nilotica* leaves, the first two eluted fractions, An1 (water fraction) and An2 (30% methanol fraction) showed the strongest activities for all three $\alpha_2$-subtypes. The affinity weakened with proceeding elution (i.e., the increase in organic solvent strength). The 100% methanol fraction showed the weakest activity and all later fractions, eluted with acetone, showed only negligible affinity. Similar trend was observed during the *P. harmala* fractionation except that Ph10 (100% methanol fraction) was weaker than that of *A. nilotica* leaves. The water and 30% methanol fractions of *P. harmala* were comparatively stronger than those of *A. nilotica*, (Table II).

Table II. Affinities ($K_i$) and corresponding antagonist potencies ($K_B$) of chromatographic factions of *P. harmala* seeds and *A. nilotica* leaves extracts. The data was obtained by displacing [$^3$H]-rauwolscine ($K_i$ determinations) or competitively inhibiting epinephrine-induced binding of $^{35}$S-GTPγS ($K_B$ determination) with six serial dilutions of the fractions.

| Fractions * | $K_i$ [μg/ml] | | | $K_B$ [μg/ml} | | |
|---|---|---|---|---|---|---|
| | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ |
| Ph1 | 4.5 | 5.2 | 7.3 | 7.7 | 12 | 4.9 |
| Ph2 | 22 | 2.9 | 4.8 | 4.3 | 13 | 2.9 |
| Ph10 | 148 | 200 | 118 | n.d. | n.d. | n.d. |
| An1 | 3.3 | 4.0 | 1.8 | 1.4 | 2.9 | 0.5 |
| An2 | 11 | 16 | 25 | 53 | 42 | 308 |
| An3 | 61 | 74 | 33 | 308 | 307 | 308 |
| An4 | 400 | 615 | 210 | n.d. | n.d. | n.d. |
| An10 | 51 | 33 | 10 | n.d. | n.d. | n.d. |

* Fractions of 80% acetone extracts of *P. harmala* seeds (Ph) and *A. nilotica* leaves (An) were obtained from Sephadex LH-20 column by elution with water (Ph1 and An1), methanol 30% (Ph2 and An2), methanol 50%, 80% (An3, An4 respectively), and acetone 100% (Ph10 and An10); n.d. = not determined.

The active fractions of *P. harmala* and *A. nilotica* were subjected to radioligand competition assays in order to estimate their binding potencies. The water fractions (Ph1 and An1), 30% methanol fractions (Ph2 and An2), and 50%, 100% methanol fractions (An3 and An10) were strongly active and completely displaced specific radioligand binding, while Ph10 showed slightly weaker activity. Among the corresponding fractions (other than the fractions mentioned above), those of *P. harmala* showed weaker (maximum specific displacement up to 60%) compared to those of *A. nilotica* (maximum specific displacement up to 90%) (Fig. 1).

Ph1 and Ph2 were further separated by collecting several sub-fractions. All water and methanol sub-fractions of Ph1 and Ph2 (obtained after re-elution with water and 5–30% methanol) were strongly active and the affinity followed the same pattern as in case of the first fractionation, (i.e., decreased with increasing strength of the organic solvent). Fractions with nominal $K_i$ values of

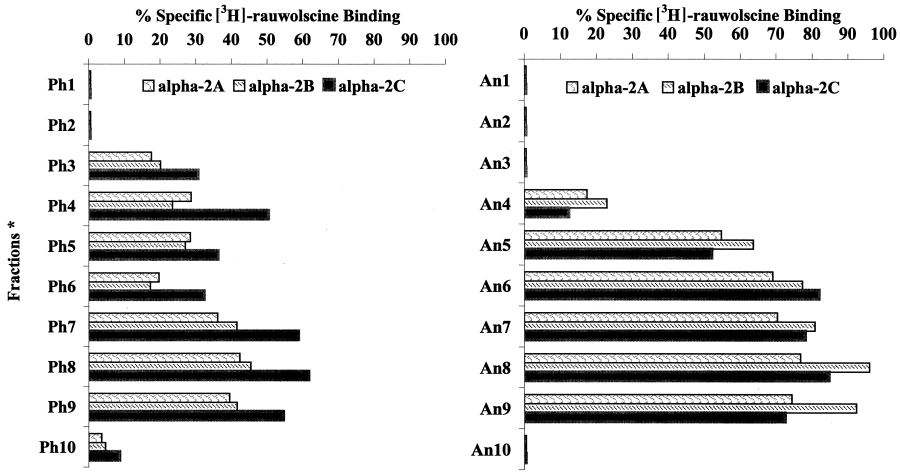


Fig. 1. Specific binding of [$^3$H]-rawolscine to alpha2-adrenergic subtypes (alpha-2A, alpha-2B and alpha-2C) in the presence of fractions (see foot note) from *P. harmala* seeds (Ph) and *A. nilotica* leaves (An) extracts.
* Fractions of 80% acetone extracts of *P. harmala* seeds (Ph) and *A. nilotica* leaves (An) on Sephadex LH-20 column were obtained by eluting with water (Ph1 and An1), methanol 30% (Ph2 and An2), methanol 50% (Ph3 and An3), methanol 80% (Ph4 and An4), acetone 20% (Ph5 and An5) acetone 40% (Ph6) and An6), acetone 60% (Phf7 and Anf7), acetone 80% (Ph8 and An8), and acetone 100% (Ph9, Ph10 and An9, An10).

Table III. Affinities ($K_i$) and corresponding antagonistic potencies ($K_B$) of sub-fractions of *P. harmala* seeds (Ph) and *A. nilotica* leaves (An) extracts. The numbers were obtained by displacing [$^3$H]-rauwolscine ($K_i$ determinations) or competitively inhibiting epinephrine-induced binding of $^{35}$S-GTPγS ($K_B$ determination) with six serial dilutions of the sub-fractions.

| | $K_i$ [μg/ml] | | | $K_B$ [μg/ml} | | |
|---|---|---|---|---|---|---|
| Sub-fractions * | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ |
| Ph1.1 | 0.9 | 0.6 | 0.9 | 0.6 | 1.4 | 0.4 |
| Ph1.2 | 0.5 | 0.6 | 1.0 | 0.8 | 2.3 | P. A. |
| Ph1.3 | 0.7 | 0.8 | 1.3 | 1.2 | ** | 1.2 |
| Ph1.4 | 4.4 | 9.7 | 17 | n.d. | n.d. | n.d. |
| Ph1.5 | 71 | 71 | 200 | n.d. | n.d. | n.d. |
| Ph2.2 | 2.3 | 2.7 | 4.2 | ** | ** | 2.1 |
| Ph2.3 | 0.8 | 0.7 | 1.3 | 2.3 | 3.3 | p.e. |
| Ph2.4 | 9.3 | 6.7 | 11 | n.d. | n.d. | n.d. |
| Ph2.5 | 103 | 58 | 138 | n.d. | n.d. | n.d. |
| An1.2 | 1.7 | 2.0 | 0.7 | 1.3 | 4.5 | 0.2 |
| An1.3 | 4.0 | 2.7 | 1.1 | 1.0 | 1.4 | 0.3 |
| An1.4 | n.a. | n.a. | n.a. | n.d. | n.d. | n.d. |
| An2.2 | 8.9 | 6.7 | 6.2 | 2.3 | 3.1 | 1.2 |
| An2.3 | 20 | 11 | 5.3 | 3.3 | 1.0 | 1.4 |
| An2.4 | 174 | 105 | 33 | n.d. | n.d. | n.d. |
| An2.5 | 200 | 308 | 58 | n.d. | n.d. | n.d. |

* Sub-fractions were obtained by purifying the water (Ph1 and An1) and methanol 30% fractions (Ph2 and An2) by eluting with water (Ph1.1-Ph2.2 and An1.1-An2.2), methanol 5% (Ph2.3 and An2.3), methanol 10% (Ph2.4 and An2.4) and methanol 15% (Ph2.5 and An2.5) on Sephadex LH-20 column; ** incomplete dose response (tested concentrations did not result in full coverage of dose response curve, i. e., the maximal effect was not well defined); n.d. = not determined; n.a. = no activity; p.e. = partial effect (the amplitude of the antagonistic effect was smaller than that of the reference antagonist rauwolscine).

above 50 μg/ml were considered negligibly active and their $K_B$ values were not determined. In general, the $K_B$ and the $K_i$ values showed good agreement (Table III). However, with fractions Ph2.2 on $\alpha_{2A}$, Ph1.3, Ph2.2 and Ph2.3 on $\alpha_{2B}$, incomplete concentration-response curves were obtained and their $K_B$ values could not be estimated. For Ph1.2 and Ph2.3, partial antagonist effects were noted. This could be due to the presence of partial agonist activities in these fractions.

*Peganum harmala* (Zygophyllaceae) is a commonly found shrub in Pakistan. As a folk medicine, the steam bath with its seeds is used in conditions of nervousness, weariness, and exhaustion (Dastur, 1970). The seeds of *P. harmala* are rich in indole alkaloids (e.g., harmaline, harman, harmine, and peganine) and a quinoline alkaloid, vasicnone (Kusmenoglu *et al.,* 1996). These alkaloids show a variety of CNS related pharmacological actions in human beings and in animals like hallucinogenicity, motor depression, cytotoxicity, CNS stimulation, and hypotension (Bolle *et al.,* 1996). Hilal *et al.,* (1979) found that harmaline and harmine are the major alkaloids in the seeds (6.75% and 4.4% respectively), while peganine (0.51%) is the major alkaloid of the leaves.

In view of the relevant pharmacological actions of glycoalkaloids, five alkaloids of *P. harmala* were tested for their binding properties on three human $\alpha_2$-adrenoceptor subtypes. Only 6-methoxyharmalan showed some moderate affinity for the $\alpha_{2A}$-adrenoceptor (530 ± 40 nM). The binding potentials of harmaline and harmine, the major alkaloids of *P. harmala* seeds, were negligible. The absence of any significant effect of these two major alkaloids indicates that the active principle in *P. harmala* proba-

Table IV. Affinity ($K_i$ in nM) of *P. harmala* alkaloids on human $\alpha_2$-adrenoceptor subtypes. The numbers were obtained by displacing $^3$H-rauwolscine with six serial dilutions of the alkaloids. [a]

| Compound | $\alpha_{2A}$ | $\alpha_{2B}$ | $\alpha_{2C}$ |
|---|---|---|---|
| Demissidine | >10000 | >10000 | >10000 |
| Harmaline | 3400 ± 900 | 4500 ± 300 | >8200 |
| Harmine | >6200 | >7000 | >10000 |
| 6-Methoxyharmalan | 530 ± 40 | 5400 ± 400 | >7000 |
| Norharmane | >11000 | >11000 | >10000 |

[a] The measurements are mean values ± SEM, $n$ = 3.

bly belongs to one or more components of the seed extracts present in lower quantities (Table IV).

*Acacia nilotica* (Mimosaceae) is a tropical tree and its beans, seeds, bark and gum are rich in tannins, related polyphenols, and phenolic acids (Devi and Parshad, 1991; Wassel *et al.,* 1990). Auriculoside, a flavan isolated from *A. auriculiformis* showed 80% CNS depressant activity *in vitro*, (Sahai *et al.,* 1980). While bark, seeds, fruits and roots of *Acacia* are widely used as folk medicines, the leaves of *A. nilotica* are not common for this purpose and their chemical composition has been relatively less investigated. Dube *et al.* (2001) determined the total phenolics and total proanthocyanidins contents and have found relatively high amounts of these chemical classes. These results for phenolic compounds are in agreement with our recent study (Saleem *et al.,* 2001). However, a possible role of the phenolic compounds for the activity of *A. nilotica* leaf extracts on $\alpha_2$-adrenoceptor remains to be determined.

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