

Full Length Research Paper

## Peganine isolated from *Anisotes trisulcus* as a smoking deterrent and anorexigenic agent

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This study concerns the discovery of the effectiveness of an alcoholic total extract of the aerial parts of the plant *Anisotes trisulcus* (Forssk.) Vahl family Acanthaceae and its active constituent peganine as a new means for suppression of tobacco intake and as a suppression to appetite (as anorexigenic). The treatment of Wistar rats with the total alcoholic extract in doses of 600 mg/100 mL drinking water decreased oral nicotine intake by 87.7% and food intake by 84.9%. The active constituent peganine in doses of 1.4 mg/mL drinking water decreased nicotine consumption by 79% and food intake by 77.8%. In other experiments in mice, the extract enhanced nicotine actions by more than 70% and mimicked nicotine in inducing intestinal stimulation in the isolated guinea-pig ileum. Peganine is hereby claimed to be a new deterrent for tobacco intake and as anorexigenic agent.

**Key words:** Peganine, *Anisotes trisulcus*, smoking deterrent, anorexigenic agent.

### INTRODUCTION

Throughout the world, almost 20% of the population enjoy tobacco actions via either smoking [cigarettes, pipes, or shisha (nargilla)] or its sublingual intake under the tongue in a semi-dry form moistened with sodium bicarbonate (or as snuff), for example, in Sudan and some other African countries (ELTahir, 1985).

Chronic consumption of tobacco can produce two types of adverse reactions, namely cancer of lungs and throats when it is smoked due to the tar components, and a group of diseases such as hypertension, arrhythmias, hyperlipidemias, asthma, and gastric and duodenal ulcers together with development of dependence with the consequence maintenance of intake (ELTahir, 1985; Taylor and Bettcher, 2000; Doll et al., 2004). These disorders usually predispose to death.

Nicotine usually mediates its actions via two types of main receptors located at two sites in the body namely

postsynaptically in the neuromuscular junction or the skeletal muscles and in the neuronal tissues peripherally and centrally at the ganglia and neurons. It excites these receptors to release acetylcholine (Sargent, 2000). The neuronal nicotinic receptors are ion-gated channels each of which is composed of 17 subunits. 10 of them are type 4, 4 are type together with  $\epsilon$ , and (epsilon) (Sargent, 2000). The most important receptor that is involved in nicotine dependence and the support of maintenance of nicotine intake in its various forms is the one containing the subunits  $\alpha 4 \beta 2$  (Cohen et al., 2003). Activation of these receptors releases dopamine via release of acetylcholine in the nucleus accumbens and the frontal cortex (Dani and Biasi, 2001). The release of dopamine plays an important part in nicotine-induced dependence, toxic actions, and addiction (Di Chiara, 2000).

Many investigators throughout the world are involved in researches aimed at discovering drugs to help in cessation of tobacco intake in its various forms. This paper describes for the first time a chemical, namely the alkaloid peganine, isolated from the aerial parts of *A. trisulcus* (Forssk.) Vahl (Acanthaceae) collected from the southern region of Saudi Arabia (Collenette, 1999) that suppresses nicotine consumption to help in the cessation of tobacco intake.

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Peganine is the major quinazoline alkaloid isolated from *A. trisulcus* (Al-Azizi, 1997). The alkaloid was previously isolated from different plant species (Grogger and Johne, 1965; Huq et al., 1967; Ghosal et al., 1975; Kurbanov and Zharekeev, 1974; Schipper and Volk, 1960). Besides peganine, the plant produced other alkaloidal constituents such as anisotine, vasicinone (Al-Azizi, 1997), trisulcusine and methoxypeganine (Al-Rehaily et al., 2002). *A. trisulcus* is a shrub locally known as Almodh (Al-Hubaishi and Muller-Hohenstein, 1984), a traditional herbal medicine in the Arabian Peninsula that found its way into the folk medicine of Saudi Arabia as an antidiabetic. The plant is also used as treatment for all hepatic conditions including hepatitis, jaundice and other hepatic problems (Fleurentin et al., 1986; Lanhers et al., 1986; Al-Harbi et al., 1992). The pharmacognostical studies on the leaf of the plant have also been conducted (Al-Rehaily, 2000). The alkaloid peganine was reported to possess abortifacient and uterine stimulant activity (Gupta et al., 1977, 1979; Chandhoke et al., 1978). In addition, it was shown to produce slight but persistent bronchodilatation, slight hypotension, and to inhibit peristalsis of isolated gut (Lahiri and Pradiian, 1964). Other studies showed bronchoconstriction and a negative inotropic effect of peganine with reduced coronary flow in the isolated heart (Lahiri and Pradiian, 1964).

## MATERIALS AND METHODS

### Plant material

*A. trisulcus* was collected in March, 2005 along the road toward Fifa Mountains, Saudi Arabia. The plant was collected by Dr. Adnan J. Al-Rehaily. The plant was identified by the taxonomist at the department of Pharmacognosy, College of Pharmacy, King Saud University (KSU). A voucher specimen was deposited at the Herbarium of the Medicinal, Aromatic and Poisonous Plants Research Center, KSU, Riyadh, Saudi Arabia.

### Extraction

The dried aerial parts (1 kg) of *A. trisulcus* were exhaustively extracted by cold percolation with 90% EtOH. The combined EtOH extract was concentrated *in vacuo* at 40°C to produce a solid residue (75 g). The alcohol extract was dissolved in 225 ml of 2% tartaric acid and extracted with  $\text{CHCl}_3$  to remove acidic and neutral components (8 g) (Fraction AT-1). The aqueous acidic solution was then alkalized with dilute  $\text{NH}_4\text{OH}$  to pH 9 and extracted with  $\text{CH}_2\text{Cl}_2$  to produce 0.688 g (Fraction AT-2). The aqueous layer was made more alkaline with  $\text{NH}_4\text{OH}$  to pH 12 and extracted with ethyl acetate to get 0.64 g (Fraction AT-3). The remaining aqueous phase was made acidic with tartaric acid to pH 4 and then lyophilized to yield 6 g (Fraction AT-4).

### Isolation and identification of peganine

The fraction AT-2 (0.688 g) was chromatographed via chromatotron 2 mm plate using EtOH absol.  $\text{CHCl}_3$ : $\text{NH}_4\text{OH}$  (5:95:4 drops) to afford 160 mg of peganine (23.26% yield) that was crystallized using mixture of  $\text{CHCl}_3$  and MeOH. The alkaloid was identified as

peganine by comparing its physical and spectral data with those reported previously in our laboratory and with the literature (Al-Azizi, 1997).

### Preparation of the alcohol extract for biological studies

The total extract was suspended in 0.25% aqueous sodium carboxymethylcellulose to give a concentration of 12.5% (w/v) initially and then diluted with the same vehicle as appropriate.

### Animals

The animals used in this study were Swiss albino mice, albino Wistar rats, and albino guinea-pigs. These were supplied by the Animals' Care Center at the College of Pharmacy, King Saud University. All animal procedures presented in this study were approved by the Ethics Committee for the use of non-human animals in biological research of the Department of Pharmacology, College of Pharmacy, King Saud University.

### Determination of lethal dose 50 (LD<sub>50</sub>) of the extract

Male Swiss albino mice (25 g body weight) and male albino Wistar rats (200 g body weight) were divided each into 5 groups (N = 10 animals per group). Animals in the different groups were injected with the extract intraperitoneally to provide doses of 0.4, 0.8, 1.6, 2.4 and 3.2 g/kg in the different groups. The animals were observed - by the naked eye - for any changes in behavior or death continuously for 2 h following the injection. Any live animals were observed for 72 h thereafter. The percentage of death in each group was calculated. The lethal dose 50 (LD<sub>50</sub>) values were then determined following the methods of Litchfield and Wilcoxon (1949), Paget (1981) and Ghosh (1984).

### Induction of nicotine convulsions

Male Albino mice were divided into different groups (N = 5 animals per group) and injected with different doses of nicotine acid tartarate dissolved in water. The animals were injected (i.p) with doses 0.8, 1.6, 3.2 and 6.4 mg/kg. The animals were continuously observed with the naked eye for 2 h for appearance of any changes in behavior such as tremors, convulsions (clonic or tonic), abnormal movements' changes in tail position and shapes, changes in locomotor activity and death.

To investigate the effect of the extract on nicotine-induced convulsions and death a sub-effective dose of the extract, (a dose of 1 g/kg) that did not produce any observable changes in behavior was initially administered to a group of 5 naive mice. Then after 5 min nicotine LD<sub>25</sub> was then administered and the animals were observed continuously for any changes in behavior, convulsions or death. The time of convulsions and death were recorded.

### Effect of the extract on isolated guinea-pig ileum

Male Albino guinea-pigs (450 g body weight) were killed using excessive ether anesthesia. The abdomen was opened and pieces of ileum (2 cm long) were cut and suspended in an organ bath containing oxygenated Krebs' solution (pH 7.4) at 37°C. One end of the tissue was attached to the bottom of the organ bath and the other end was attached to an isometric transducer (Myograph F60 with 0.5 and 5 g tension calibrations, supplied by Narco Biosystems, Houston, USA) and attached to a universal coupler No. 7189 fitted into a Narco physiograph. To examine the effect of the

extract, the latter was added to the fluid bathing the tissue in different doses and allowed to contact the tissue for 45 sec. The produced effect was recorded. To examine the effect of the blockers on the induced contraction, the following procedure was used. Hexamethonium (at a dose of 10-100 g/mL) or atropine (at a dose of 0.1-20 g/mL) was added to the bathing fluid and allowed to contact the tissue for 5 min and then the submaximal dose of the extract was added. The percentage inhibition induced by the blocker on the extract-induced contractions was then calculated.

#### **Effect of the total extract, its fractions and peganine on nicotine and food consumption in rats**

Male Wistar rats (200 g body weight) were divided at random into various groups (N = 6 animals per group). Each animal was placed in a stainless steel cage and provided with a known weight of normal rat chow food pellets and drinking bottle containing a known volume of aqueous 0.025% sodium carboxymethylcellulose alone or containing either nicotine acid tartarate to provide a final concentration of 40 g/mL (in case of nicotine control group) or the same concentration of nicotine plus the indicated concentrations of the whole extract, one of its fractions or peganine. The latter were added in form of their emulsions in 0.25% aqueous sodium carboxymethylcellulose in volumes that provide a final concentration of 0.025% of sodium carboxymethylcellulose in the drinking fluid. The animals were allowed both food and the drinking liquid *ad libitum*. The room temperature where the animals were placed was maintained at  $23 \pm 2^\circ\text{C}$ . The light cycle was kept at 12 h light and 12 h dark. The relative humidity was maintained at  $75 \pm 5\%$ . Both food and drinking liquid consumptions were measured every 2 days and at the end of the treatment which continued for complete seven consecutive days. New solutions were provided every two days. The influence of each treatment on food consumption, the drinking fluid intake and hence nicotine consumption was calculated at the end of the treatment.

#### **Statistical analysis**

All results were reported as their means  $\pm$  s. e. mean with N = number of experiments. Statistical significance between control and treated groups were performed using non-paired *t*-test or analysis of variance (ANOVA) as appropriate.

## **RESULTS**

### **Effects of the total alcohol extract**

- A) Lethal Dose 50 (LD<sub>50</sub>): in rats and mice was 1.8 g/kg (i.p).
- B) Lethal Dose (LD<sub>100</sub>) in rats and mice was more than 2.4 g/kg (i.p).  
Symptoms before death:
  - 1- Increase in locomotor activity
  - 2- Abdominal respiration (shallow respiration)
  - 3- Shaking of tail
  - 4- Convulsions: Onset of death following administration was 4-5 min.
- C) Effect of the extract on nicotine-induced convulsions and death in mice. Table 1 shows the central actions of nicotine alone in the dose range 1.6 to 6.4 mg/kg (i.p) in mice and the effect of the total extract in a

sub-effective dose of 1 g/kg (i.p) on nicotine-induced actions. As it can be seen in Table 1, the extract at a dose of 1 g/kg (i.p) into mice potentiated the actions of nicotine, namely convulsions, by 300%.

- D) Effect of the total extract on the isolated guinea-pig ileum
  - 1- Exposure of the guinea-pig ileum to the total extract in doses of 15, 30 and 60 g/mL bathing fluid induced dose-dependent contractions.
  - 2- The stimulant effect was not blocked by either hexamethonium (100 g/mL) or atropine (10 g/mL).

In summary, the stimulant effect of the extract was not due to activation of ganglia or muscarinic M<sub>3</sub> receptors.

### **Effect of the total alcohol extract on nicotine and food consumption in rats**

In control rats provided with drinking water containing nicotine in a concentration of (40 g/mL), the daily consumption of the drinking vehicle per 200 g rat was  $26.1 \pm 1.3$  ml and the daily food consumption was  $23.9 \pm 0.9$  g. Treatment of the animals with the total alcoholic extract in a dose of 600 mg/100 mL drinking vehicle reduced the nicotine consumption and food intake by 87.7 and 84.9%, respectively. The cumulative effects are shown in Table 2.

### **Effect of the different fractions of the total alcohol extract on nicotine and food consumption in rats**

Treatment of rats with each of the different fractions of the total alcohol extract mixed with nicotine in the drinking vehicle revealed that only fraction AT-2 possessed some ability to suppress both nicotine and food intake. Table 3 shows the cumulative results. When higher concentrations of the different fractions were used, here again fraction AT-2 proved to be the most effective and produced significant reductions in both actions (Table 4).

### **Effect of peganine on nicotine and food consumption in rats**

As peganine was found to be the major constituent from the fraction AT- 2 in this study, it was tested in rats. Treatment of the animals with peganine 1.4 mg/mL in the drinking vehicle mixed with nicotine (40 g/mL) produced significant reduction in both nicotine and food consumptions. It decreased the nicotine consumption and food intake by 79 and 77.8%, respectively. The details are shown in Table 5.

The effective dose 50 values (ED<sub>50</sub>) that produce 50% inhibitions regarding nicotine and food consumptions were  $24.3 \pm 1.7$  and  $25.1 \pm 0.9$  mg/kg orally, respectively.

**Table 1.** Effect of the total extract on nicotine-induced actions in mice.

S/N	Treatment	Action
1	Nicotine 1.6 mg/kg (i.p)	Increase in locomotor activity Raising of tail Tremors Shaking of the body Increase in respiratory rate No death
2	Nicotine 3.2 mg/kg (i.p)	Tremors Fanning of tail Convulsions (Clonic-type) Tremors Salivation No death
3	Nicotine 6.4 mg/kg (i.p)	Circular movements Convulsions Death after 3 minutes
4	Total extract 1 g/kg (i.p) for 5 min	No clear actions
5	Total extract 1 g/kg (i.p) + nicotine 1.6 mg/kg (i.p)	Rapid respiration Tremors Shaking of tail Convulsions Loss of righting reflex Death after 3 minutes

**Table 2.** Effect of total alcoholic extract on nicotine and food consumption rats (N = 6 per group).

Treatment	Concentration of the extract in drinking water (mg/100 mL)	Quantity of extract consumed daily per 200 g rat (mg <sup>a</sup> )	Concentration of nicotine in drinking water (g/mL)	Liquid consumed daily per 200 g rat per day (mL)	Quantity of nicotine consumed per day (mg)	% decrease	Quantity of food consumed per 200 g rat per day (g)	% decrease
Control	-	-	40	26.1 ± 1.3	1.04	-	23.9 ± 0.9	-
Total alcoholic extract	600	19.2	40	3.2 ± 0.2 <sup>*</sup>	0.128	87.7	3.6 ± 0.12 <sup>*</sup>	84.9

<sup>a</sup>Calculated from the daily consumed liquid \* p < 0.01, N = 6.

**Table 3.** Effect of the different fractions of the total extract on nicotine and food consumption in rats.

Treatment	Concentration of the extract in drinking water <sup>a</sup> (mg/100 mL)	Quantity of extract consumed daily per 200 g rat <sup>b</sup> (mg)	Concentration of nicotine in drinking water (µg/mL)	Liquid consumed daily per 200 g rat (mL)	Quantity of nicotine consumed per day (mg)	% decrease	Quantity of food consumed per 200 g rat per day (g)	% change
Control	-	-	40	26.7 ± 1.1	1.068	-	24.9 ± 0.9	-
Fraction AT-1	64	16.64	40	26 ± 0.6	1.04	2.7	24.3 ± 1.3	2.4
Fraction AT-2	5.5	1.38	40	25.2 ± 1.2	1.008	5.6	24 ± 1.2	3.6
Fraction AT-3	2	0.524	40	26.2 ± 0.4	1.048	1.87	25.1 ± 1.7	-( 0.8)
Fraction AT-4	48	12.67	40	26.4 ± 0.8	1.056	1.12	25.5 ± 1.4	-( 2.4)

<sup>a</sup> Calculated and chosen in relation to the % of the fraction in the total extract.

<sup>b</sup> Calculated with reference to the daily consumption.

**Table 4.** Effect of higher concentrations of the different fractions of the total Extract on nicotine and food consumption in rats.

Treatment	Concentration of the extract in drinking water (mg/100 mL)	Quantity of extract consumed daily per 200 g rat <sup>b</sup> (mg)	Concentration of nicotine in drinking water (µg/mL)	Liquid consumed daily per 200 g rat (mL)	Quantity of nicotine consumed per day (mg)	% decrease	Quantity of food consumed per 200 g rat per day (g)	% change
Control	-	-	40	26.7 ± 0.9	1.068	-	24.9 ± 0.3	-
Fraction AT-1	330	84.15	40	25.5	1.02	4.5	23.2 ± 0.2	6.8
Fraction AT-2	450 <sup>a</sup>	75.60	40	16.8 ± 0.2 <sup>*</sup>	0.672	37.1	18.1 ± 0.1 <sup>*</sup>	27.3
Fraction AT-3	230	59.56	40	25.9 ± 1.3	1.036	3	27.2 ± 0.6	-( 9.2)
Fraction AT-4	360	93.96	40	26.1 ± 1.7	1.044	2.2	26.9 ± 0.4	-( 8)

<sup>a</sup> From Table 3, fraction AT-2 seemed to contain the highest activity. Thus, a dose equivalent to 75% of the effective dose of the total extract was chosen and tried (Table 2).

<sup>b</sup> Quantity was calculated by reference to the daily drinking liquid consumption.

<sup>\*</sup> p < 0.05, N = 6.

**Table 5.** Effect of peganine (vasicine) on nicotine and food consumption in rats.

Treatment	Concentration of peganine in drinking water (mg/100 mL)	Quantity of peganine consumed daily per 200 g rat (mg)	Concentration of nicotine in drinking water (g/mL)	Liquid consumed daily per 200 g rat per day (mL)	Quantity of nicotine consumed per day (mg)	% decrease	Quantity of food consumed per 200 g rat per day (g)	% decrease
Control	-	-	40	26.25 ± 0.9	1.05	-	25.5 ± 1.4	-
peganine	140	7.7	40	5.5 ± 0.13	0.22	79	5.66 ± 0.3	77.8

p < 0.01, N = ??? (Please provide the value for N)

The minimum inhibitory concentrations of peganine (MIC) (10%) on nicotine and food

consumptions were 4.6 ± 0.08 and 5.1 ± 0.2 mg/kg orally, respectively. The lethal dose 50

(LD<sub>50</sub>) of peganine in rats was 189 ± 10.1 mg.kg orally.

## DISCUSSION

The results of this study revealed that the extract of *A. trisulcus* induced significant inhibition in nicotine consumption, reduced the lethal dose of nicotine by 75% and mimicked nicotine in inducing intestinal contractions (probably via activation of a subtype of nicotine receptors in the guinea-pig intestinal ganglia-albeit not blocked by the ganglionic blocker hexamethonium). Furthermore, it induced significant suppression of food intake. Its active constituent peganine (vasicine or linarine) which is known chemically as 1,2,3,9-tetrahydropyrido[2,1- b]quinazolin-3-ol produced potent significant inhibitions of nicotine intake and food consumption. It exerted a potent anorexigenic action. In fact this constituent acted as a nicotine substitute.

Peganine actions are probably mediated via activation of  $\alpha_2$  nicotinic receptors as does nicotine leading to release of dopamine in the mesolimbic sites of the brain. Thus, it probably acted as a nicotine substitute leading to satisfaction of the rewarding system leading to an explanation of the reduced nicotine intake (Tapper et al., 2004).

Generally, speaking an agonist at a certain receptor may either be a potent, low potent partial or potent partial agonist. Previous pharmacological studies revealed that potent nicotinic agonists or low potency partial agonists would not be expected to antagonize the effects of nicotine but agents that act as potent partial agonists can antagonize nicotine (Stuhmer, 1992; Coe et al., 2005). For instance varenicline, the potent partial nicotinic  $\alpha_2$  agonist, in a dose of 5.6 mg/kg significantly blocked nicotine (1 mg/kg)-induced increase in dopamine turnover in the mesolimbic system of rats (Coe et al., 2005). Thus, since the extract containing peganine did not antagonize nicotine-induced convulsions and death but instead mimicked nicotine and reduced its LD<sub>50</sub> by > 50% and in addition it substituted for nicotine in the nicotine drinking experiments it is plausible to suggest that peganine acted as does nicotine at the  $\alpha_2$  nicotinic receptor. Indeed, the types of convulsions observed before death following peganine were reminiscent to those of nicotine.

Thus, it is highly likely that peganine can act to limit craving and withdrawal from nicotine in chronic tobacco consumers (smokers or snuff intakers). Thus, we claim that peganine has the potential of a novel treatment for tobacco dependence. In this aspect, it is hoped to be more fruitful than the partial nicotinic agonist (-)-cytisine (Barlow and McLeod, 1969; Scharfenberg et al., 1971) that has poor blood brain barrier penetrability (Reavill et al., 1990). It is hoped to be a new addition to the armamentarium of tobacco cessation campaigns that include the skin patch containing both nicotine and its antagonist mecamylamine (Rose et al., 1994), the varenicline, the partial  $\alpha_2$  nicotinic agonist (Coe et al., 2005), the antidepressant bupropion (or amfebutamine) (Warner and Shoab, 2005) and the nicotine conjugate vaccine (Nicvax®) (Fattom et al., 2003).

Considering food intake and appetite, we can recall that the two most important brain neurotransmitters that suppress food intake are dopamine and serotonin (Silverstone, 1992). The significant inhibitions induced by both extract and its constituent peganine in food intake incline us to believe that the anorexigenic effects were mediated by dopamine release via initial activation of  $\alpha_2$  nicotinic receptors. Indeed activation of these receptors releases dopamine (Dani and Biasi, 2001; Di Chiara, 2000; Tapper et al., 2004).

## Conclusion

Both extracts of *A. trisulcus*, and its constituent peganine, possessed properties that enabled them to suppress nicotine consumption and food intake.

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