Arab Gulf J. Scient. Res. **13**(1), pp. 187-198 (1995)

Effects of Pipe Tobacco Extract on Locomotor Activity and Brain Acetylcholinesterase Level in Mice

J.S. Ajarem and M.Ahmad

Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT. The effects of pipe tobacco extract on locomotory behaviour and brain acetylcholinesterase (AChE) activity were investigated in groups of male Swiss-Webster albino mice injected subcutaneously with 5, 10 or 20 mg/kg body weight in aliquotes of 0.1 ml normal saline. The behavioural indices namely numbers of squares crossed, squats as well as durations of locomotion and immobility were observed at 15, 30, 60 and 120 min post injection (PI) . The mice were also allowed to cling to a wire and the time taken to fall down (clinging time) was used as an indication of myorelaxation. The brain AChE level was estimated at 30 and 120 min PI. A significant, dose-dependent suppressive effect of the extract was observed on all tested behavioural indices. The brain AChE activity was significantly elevated at lower doses, whereas it was initially inhibited with the high dose of 20 mg/kg body weight before returning to baseline. There seems to be some sort of correlation between the behavioural indices tested and brain AChE activity due to tobacco extract treatment.

The use of tobacco by humans (in any form) is crucially related to nicotine addiction, at least as intransigent as that produced by other commonly abused drugs (Jorenby *et al.* 1990). However, tobacco is ragarded as a valuable aid for persons treated for opioid dependence (Blumford *et al.* 1974, Haertzen *et al.* 1983 and Henningfield 1984). Moreover, people do not habitually smoke if nicotine is not present in cigarettes (Ashton and Stephney 1982). Nicotine appears to act at the neuromuscular junction, as well as at the autonomic ganglia and in the brain. Such

multiplicity of effects makes it difficult to predict the exact responses to nicotine (Marks *et al.* 1983 a) but its inherent properties have made it a classical tool in pharmacological investigations (Holmstedt 1988). In an attempt to clarify the situation several workers have used rodent models for studying the effects of nicotine on behaviour (Schechter and Rosecrans 1972, Stolerman *et al.* 1973, 1974, Hubbard and Gohd 1975, Hatchell and Collins 1977, Baer *et al.* 1980, Johns *et al.* 1982 and Sorenson *et al.* 1991).

Pouch tobacco is widely used by pipe smokers and may contain several chemical substances in addition to tobacco such as glycerol, licorice, sorbitol and invert sugars. However, nicotine forms the primary pharmacologically active and addictive agent in the tobacco (Taylor 1980 and Anon 1988). Though many studies have been carried out with pure nicotine in animal models, yet little is known about the behavioural and biochemical effects of total extracts of pipe tobacco.

Thus, the present study examined the effects of pipe-tobacco extracts on locomotory behaviour, as well as on brain acetylcholinesterase (AChE) activity in male mice. correlations between the two indices were sought.

Materials and Methods

Animals

Swiss-Webster albino mice (*Mus musculus*), were used in the present study. Adult male mice, of the same age and weight, were housed 6 per cage in plastic cages measuring 30 x 12 x 11 cm, in an environmentally controlled room with a temperature of 20-22 °C and a 12-hour light /dark cycle. Unless otherwise stated, Pilsbury's mouse food and water were provided *ad libitum.*

Tobacco Extract

The commercially available pouch pipe-tobacco was purchased from the local market in Riyadh. A known weight of this tobacco was mixed with a known volume of normal saline and thoroughly stirred with an electro-magnetic stirrer overnight at room temperature. Subsequently, the mixture was filtered under a slow vacuum and the filtrate was adjusted such that a constant injectable volume (0. 1 ml) contained 5, 10 or 20 mg of the tobacco extract kg^{-1} mouse body weight. The exact nicotine content in the extract was not determined. Each dose was injected subcutaneously and behavioural indices were assessed at 15, 30, 60 and 120 min intervals post injection (PI). Control animals were injected with 0. 1 ml normal saline only.

Brain Acetylcholinesterase Activity Determination

Twenty four mice (six per group) were used for enzyme estimation. Each group was divided into two sub-groups with 3 animals in each. All animals were killed by decapitation 30 and 120 min PI and their brains were immediately removed, weighed

and frozen. A 10% (w/v) homogenate of brain tissues was prepared in phosphate buffer (0.067 M, pH 7.2) as described by Ajarem and Ahmad (1991). The AChE activity was estimated by the method of Hestrin (1949) and expressed as µmoles acetylcholine chloride hydrolysed mg^{-1} wet tissue weight $hour^{-1}$ at 37 ± 1 °C.

Behavioural Tests

Following injections, the mice were placed in experimental wooden arena for observations at 15, 30, 60 and 120 min time intervals.

The wooden arena measured 80 x 80 x 30 cm and the floor was divided into 64 equal sized squares. Various behavioural "elements" were observed as described by Ajarem (1987). Elements of locomotor activity included on the numbers of squares crossed, numbers of squats, as well as durations of locomotion and immobility. The observations in the arena lasted 300 seconds for each animal and seven animals were used in each group.

Myorelaxation Test

A separate group of mice (seven per group) were allowed to cling to a hanging wire 15, 30, 60 or 120 min PI and the period until they dropped was recorded. This recorded clinging time was taken as a measure of the time for the animal's muscle relaxation. The wire was kept one foot above the working table surface so as to avoid injury to the animals during their fall.

Statistical Analysis

The data were statistically analysed by the two-way ANOVA using Minitab programme, followed by Student's t-tests (Yamane 1973).

Results

The results for numbers of squares crossed in the different treatment groups are given in Table 1. The ANOVA revealed that the tobacco extract has significantly reduced the number of squares crossed by the mice, an effect which is time - and dose-dependent ($F\{9,96\} = 2.70$, $p<0.05$). Treatment also reduced the number of squats shown by the animals (Table 2) except that this effect is only significant ($F\{9,96\} = 2.70$ $p<0.05$) at the higher doses. On the other hand, a dose-and time-dependent, statistically significant ($F\{9,96\} = 2.51$, $p<0.01$) gradual decrease is observed in the locomotory duration of the treated mice (Table 3) . In contrast, the duration of immobility was significantly ($F\{9,96\} =3.71$, $p<0.01$) increased in a time- and dose-dependent fashion (Table 4). A significant ($F\{9,96\} = 3.31$, $p<0.01$) time- and dose-dependent decrease was also observed in the clinging time PI, indicating a reduction in the time taken for myorelaxation (Table 5).

At the lower doses (5 and 10 mg/kg body weight) of the extract, AChE activity

Table 1. Median values (with ranges) for the effect of tobacco extract on the number of squares crossed by male mice

Doses of pipe tobacco extract (mg/kg body weight)	Post injection time (minutes)			
	15	30	60	120
Control	235.5 (190-327)	192 (153-280)	114.5 (88-161)	69 (58-152)
5	260.5 (223-453)	230.5 (170-319)	86* (33-156)	26.6* (19-102)
10	168.5* (117-362)	73** (42-169)	32.5** (12-142)	14** (8-39)
20	96.5*** (64-221)	32.5*** (25-131)	27.5*** (16-76)	3*** (0-24)

* P < 0.05 as compared to control
** P < 0.01 as compared to control
*** P < 0.005 as compared to control

Table 2. Median values (with ranges) for the effect of tobacco extract on the number of squats by male mice

Doses of pipe tobacco extract (mg/kg body weight)	Post injection time (minutes)			
	15	30	60	120
Control	2.5 (0 - 4)	3 (1 - 4)	3 (2 - 5)	5 (2 - 6)
5	0.5 (0 - 3)	1.5 (1 - 2)	2 (1 - 4)	3.5 (1 -5)
10	1 (1 - 4)	0.5* (1 - 3)	1* (1 - 2)	1.5* (1 -2)
20	1* (0 - 2)	1.5* (1 - 2)	1* (1 - 1)	1* (1 - 2)

* P < 0.05 as compared to control

Table 3. Median values (with ranges) for the effect of tobacco extract on duration of locmotion (seconds) in male mice

Doses of pipe tobacco extract (mg/kg body weight)	Post injection time (minutes)			
	15	**30**	**60**	**120**
Control	242.5 (200-300)	210 (120-275)	172.5 (50-220)	115 (55-175)
5	295 (125-300)	250 (55-285)	110* (15-205)	45 (35-130)
10	222.5* (110-275)	140** (30-190)	92.5** (5-130)	12.5*** (10-50)
20	130.5** (45-225)	35*** (15-110)	10*** (0-60)	5*** (0-45)

* $P < 0.05$ as compared to control
** $P < 0.01$ as compared to control
*** $P < 0.005$ as compared to control

Table 4. Median values (with ranges) for the effect of tobacco extract on the immobility duration (seconds) in male mice

Doses of pipe tobacco extract (mg/kg body weight)	Post injection time (minutes)			
	15	**30**	**60**	**120**
Control	57.5 (0 -100)	90 (25-180)	127.5 (80-250)	185 (125-245)
5	5 (0 - 175)	50 (15-245)	190 (95-285)	255.6* (170-265)
10	77.5* (25-190)	160** (10-270)	207.5* (170-295)	287.5** (250-290)
20	170** (75-255)	265.5** (190-285)	290.5** (240-300)	295** (255-300)

* $P < 0.05$ as compared to control
** $P < 0.01$ as compared to control

Table 5. Median values (with ranges) for the effect of tobacco extract on the clinging time (seconds) in male mice

Doses of pipe tobacco extract (mg/kg body weight)	Post injection time (minutes)			
	15	**30**	**60**	**120**
Control	21.5 (14-85)	34 (22-175)	49.5 (22-110)	90 (60-195)
5	19.5 (15-165)	41 (9-95)	86.5 (8-115)	34* (0-170)
10	18.5* (11-110)	18.5* (14-30)	19* (13-36)	22** (17-68)
20	20.5* (7 - 37)	26* (11-50)	23.5** (7-74)	34** (21-145)

* $P < 0.05$ as compared to control
** $P < 0.01$ as compared to control

Table 6. Effect of pipe tobacco extract on the acetylcholinesterase (AChE) activity in the brain tissue of male mice

Doses of pipe tobacco extract (mg/kg body weight)	AChE activity in brain tissue ± SEM, post injection		Percent change in activity	
	30 min	**120 min**	**30 min**	**120 min**
Control	655.7±57.1	595.5 ±28.7	—	—
5	866.7*±45.6	918.3**±43.8	+32.2%	+54.2%
10	1171.6**±218.6	1562.5***±220.4	+78.7%	+162.4%
20	506.6*±78.9	609.8 ±110.4	-22.7%	+2.4%

* $P < 0.05$ as compared to control
** $P < 0.01$ as compared to control
*** $P < 0.005$ as compared to control

a Activity expressed into μ moles acetylcholine hydrolysed/g wet weight tissue/hour at 37 ± 1°C.

was significantly ($F\{9,96\} = 4.23$, $p < 0.05$) increased, but at the highest dose (20 mg/kg body weight) the activity was inhibited at 30 min PI and barely returned to normal levels at 120 min PI (Table 6).

Discussion

Pipe- tobacco extract clearly influenced normal locomotory activity of male mice in a time- and dose-dependent fashion. The overall locomotor activity decreased with increasing dose and extended time intervals PI. The lowest dose (5 mg/kg) initially caused a stimulatory effect in all behavioural activities during the first 15 to 60 min, thereafter a suppressive effect was observed at 120 min PI . In contrast the higher doses (10 and 20 mg/kg) resulted in a gradual suppressive effect over the total period of 120 min PI. This may be of importance from the pharmacokinetic point of view. The actions of nicotine have long been known to be biphasic where low concentrations are stimulatory, but higher ones, though initially stimulatory, later inhibit (Marks *et al.* 1983b). Moreover, the present study has shown that the lowest dose (5mg/ kg) has a biphasic effect, but higher ones (10 and 20 mg/kg) are inhibitory. This may be due to the variation in the form of nicotine used in either study, pure nicotine and total pipe-tobacco extract. Also, mouse strain variations could be another factor, since different strains of mice are known to respond differently to nicotine in behavioural studies (Marks *et al.* 1983a). Moreover, nicotine is also known to have suppressive effects on behaviour, as well as on various other forms of locomotory activities both in humans and experimental animals (Schechter and Rosecrans 1972, Stolerman *et al.* 1973, 1974, Russell 1979, Hatchell and Collins 1980, Henningfield and Goldberg 1983, Goldberg and Spealman 1983, Marks *et al.* 1983a, and Mundy and Iwamoto 1988).

Nicotine appears to act at the neuromuscular junctions, at the autonomic ganglia and in the brain (Marks *et al.* 1983 a). Thus, such activities could well be correlated with AChE activity. However, at low (5 mg/ kg) and medium (10 mg / kg) doses, the AChE level is elevated in a time- and dose-dependent manner, but the highest dose (20 mg / kg) is initially inhibitory, then in about two hours PI the normal level is restored. This might be due to the fast elimination of nicotine administered subcutaneously from the blood into the brain that peaked in 10-15 min (Martin *et al.* 1983), leading to the observed inhibition of AChE activity. The level of nicotine in the brain then declines over the next 50 min allowing the level of the activity of the enzyme to revert to normal. Thus this state of affairs does not seem to correlate well with the behavioural changes observed in the present study. This low correlation between the behavioural and brain AChE responses elicited by nicotine may be attributed to any, or to a combination of several factors including the form of nicotine administered, the way in which the behavioural responses are measured and the location of the site controlling the behavioural responses studied. The crude extract of pipe-tobacco as used in the present study, certainly contains substances

other than nicotine that might have some effects of their own or in combination. Thus, the effects observed in the present study may be due to the nicotine itself present in the extract or may be due to the combination of nicotine with other additives in it. However, further studies are in progress in our laboratory to evaluate those effects in mice using nicotine antagonists.

The behavioural responses were measured on whole animals, while the AChE activity was assessed in the brain only. Hence, these might not correspond directly to each other, since nicotine is known to influence the release of catecholamines both in the central (Arqueros *et al.* 1978, Giorguieff-Chesselet *et al.* 1979, and Yoshida *et al.* 1980) as well as in the peripheral nervous system (Kirpekar *et al.* 1980). However, variatios in the behavioural and biochemical responses assessed in the present study might have well been substantially affected by differences in the release mechanisms of these neurotransmitters. Moreover, such activities of nicotine on the nervous system, particularly peripheral actions at the neuromuscular junction might explain some of the reported behavioural responses *i.e.* the myorelaxation performance. Hence, the careful analysis of the role of these neurotransmitters on the peripheral nervous system would be of great utility in understanding interactions between behavioural and biochemical changes.

Acknowledgements

The authors are grateful to Professor H. S. Hussein of this department, for his critical evaluation of the manuscript and helpful suggestions and to Dr. Shafeeq Ahmed of Department of Statistics, for his help in statistical analyses.

References

Ajarem, J.S. (1987) Studies on the effect of alcohol on locomotor activity and immobility in male mice. *Proc. Saudi Biol. Soc.*, **10:** 97-104.

Ajarem, J.S. and **Ahmad, M.** (1991) Behavioral and biochemical consequences of perinatal exposure of mice to instant coffee: a correlative evaluation. *Pharmacol. Biochem. Behav.*, **40:** 847-852.

Anon. (1988) *The Health Consequences of Smoking : Nicotine Addiction.* A report of the Surgeon General, US Government printing office, Washington, D.C.

Arqueros, L., Naquira, D. and **Zunino, E.** (1978) Nicotine-induced release of catechol amines from rat hippocampus and striatum. *Biochem. Pharmacol.*, **27:** 2667-2674.

Ashton, H. and **Stepney R.** (1982) *Smoking : Psychology and Pharmacology.* Tavistock, London.

Baer, D.S. , McClearn, G.E. and **Wilson, J.R.** (1980) Effects of chronic administration of tobacco smoke to mice: Behavioural and metabolic measures. *Psychopharmacology*, **67:** 131-137.

Blumford, H.H., Cohen, S.D., Dronfield, B.E., Mordecai, E.A. , Roberts, J.C. and **Hawks, D.** (1974) British opiate users: I. People approaching London drug treatment centres, *Int. J. Addict.*, **9:** 1-23.

Giorguieff- Chesselet, M.F., Kemel, M.L., Wandscheer, D. and **Glowinski, J.** (1979) Regulation of dopamine release by presynaptic nicotinic receptors in rat striatal slices: Effect of nicotine in low concentrations. *Life Sci.*, **25:** 1257-1262.

Goldberg, S.R. and **Spealman, R.D.** (1983) Suppression of behavior by intravenous injections of nicotine or by electric shocks in squirrel monkeys: Effects of chlordiazepoxide and mecamylamine. *J. Pharmacol. Exp. Ther.*, **224:** 334-340.

Haertzen, C.A., Kocher, T. R. and **Mayasato, K.** (1983) Reinforcement from the first drug experience can predict later drug habits and / or addiction: results with coffee, cigarettes, alcohol, barbiturates, minor and major tranquilizers, stimulants, marijuana hallucinogens, heroin, opiates and cocaine. *Drug Alcohol Depend.*, **11:** 147-165.

Hatchell, P.C. and Collins, A.C. (1977) Influences of genotype and sex on behavioural tolerance to nicotine in mice. *Pharmacol. Biochem. Behav.*, **6:** 25-30.

Hatchell, P.C. and **Collins, A.C.** (1980) The influence of genotype and sex on behavioural tolerance to nicotine. *Psychopharmacology*, **71:** 45-49.

Henningfield, J.E. and **Goldberg, S.R.** (1983) Control of behavior by intravenous nicotinic injections in human subjects. *Pharmacol. Biochem*, Behav., **19:** 1021-1026.

Hestrin, S. (1949) The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. *J. Biol. Chem.* , **180:** 249-261.

Holmstedt, B. (1988) Toxicity of nicotine and *related compounds, In:* **Rand, M.J.** and **Thurau, K.** (eds), *The Pharmacology of Nicotine.* IRL Press,Oxford, Washington DC, 61-88 pp.

Hubbard, J.E. and **Gohd, R.S.** (1975) Tolerance development to the arousal effects of nicotine. *Pharmacol. Biochem. Behav.*, **3:** 471-476.

Johns, J.M., Louis, T.M., Becker, R.F. and **Means, L.W.** (1982) Behavioural effects of prenatal exposure to nicotine in guinea pigs. *Neurobehav. Toxicol. Teratol.*, **4:** 365-369.

Jorenby, D.E., Steinpreis, R.E., Sherman, J.E. and **Baker, T.B.** (1990) Aversion instead of preference learning indicated by nicotine place conditioning in rats. *Psychopharmacology,* **101:** 533-538.

Kirpekar, S.M., Garcia, A.G. and **Prat, J.C.** (1980) Action of nicotine on sympathetic nerve terminals. *J. Pharmacol. Exp. Ther.*, **213:** 133-138.

Marks, M.J. and **Collins, A.C.** (1982) Characterization of nicotine binding in mouse brain and comparison with the binding of α– bungarotoxin and quinuclidinyl benzilate. *Mol. Pharmacol.*, **22:** 554-564.

Marks, M.J., Burch, J.B. and **Collins, A.C.** (1983a) Genetics of nicotine response in four inbred strains of mice. *J. Pharmacol. Exp. Ther.*, **226:** 291-302.

Marks, M.J., Burch, J.B. and **Collins, A.C.** (1983b) Effects of chronic nicotine infusion on tolerance development and nicotine receptors . *J. Pharmacol. Exp. Ther.*, **226:** 817-825.

Martin, B.R., Tripathi, H.L., Aceto, M.D. and **May, E.I.** (1983) Relationship of the biodisposition of the stereoisomers of nicotine in the central nervous system to their pharmacolgical actions. *J. Pharmacol. Exp. Ther.*, **226:** 157-163.

Mundy, W.R. and **Iwamoto, E.T.** (1988) Actions of nicotine on the acquisition of an autoshaped lever-touch response in rats. *Psychopharmacology*, **94:** 267-274.

Russell, M.A.H. (1979) Tobacco dependence : is nicotine rewarding or aversive? *In:* **Kraonegor, N.A**. (ed), *Cigarette Smoking as a Dependence Process*, US Government Printing Office, Washington DC, 100-122 pp.

Schechter, M.D. and **Rosecrans, J.A.** (1972) Behavioral tolerance to an effect of nicotine in the rat. *Arch . Int. Pharmacodyn.Ther.*, **195:** 52-56.

Sorenson, C.A., Raskin, L.A. and **Suh, Y.** (1991) The effects of prenatal nicotine on radial-arm maze performance in rats. *Pharmacol. Biochem. Behav.* , **40:** 991-993.

Stolerman, I.P., Fink, R. and **Jarvik, E.** (1973) Acute and chronic tolerance to nicotine measured by activity in rats. *Psychopharmacologia*, **30:** 329-342.

Stolerman, I.P. , Bunker, P. and **Jarvik, M.E.** (1974) Nicotine tolerance in rats: Role of dose and dose interval. *Psychopharmacologia,* **34:** 317-324.

Taylor, P. (1980) Ganglionic stimulating and blocking agents, in: **Gilman, A.G., Goodman, L.S.** and **Gilman, A. (eds)**, *The Pharmacological Basis of Therapeutics.* 6th edn. Macmillan, New York.

Yamane, T. (1973) The t distribution, in: **Yamane, T.** (ed), *Statistics, An Introductory Analysis,* 3rd edn, Harper and Row, London.

Yoshida, K., Kato, Y. and **Imura, H.** (1980) Nicotine - induced release of nonadrenalin from hypothalamic synaptosomes. Brain Res., **182:** 361-368.

(Received 25/05/1993;
in revised form 23/03/1994)

دراسة تأثير مستخلص التبغ على السلوك الحركي وعلى نشاط أنزيم الاسيتايل كولين أستريز لذكور الفئران المخبرية

جمعان سعيد عجارم و محمد أحمد

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود - الرياض
ص .ب (٢٤٥٥) - الرياض ١١٤٥١ - المملكة العربية السعودية

لقد تم تعريض ذكور الفئران المخبرية السويسرية لتركيزات مختلفة من مستخلص التبغ هي ٥ ، ١٠ ، ٢٠ ملجم/ كجم/ وزن الجسم بالاضافة إلى الكنترول وذلك بحقنها مباشرة تحت الجلد ، وأختبر سلوكها في المنطقة المفتوحة بعد ١٥ ، ٣٠ ، ٦٠ ، ١٢٠ دقيقة من بداية الحقن وكانت مدة الاختبار لكل حيوان خمس دقائق . أما قدرة إنقباض وإنبساط عضلات الحيوان فقد اختبرت بعد الانتهاء من اختبار سلوك الحيوان مباشرة وذلك بجعله يتعلق في سلك معدني ثم يتم حساب الزمن الذي يأخذه في التعلق حتى السقوط . لقد درس نشاط أنزيم الاسيتايل كولين أستريز في المخ بعد ٣٠ ، ١٢٠ دقيقة من بداية الحقن .

النتائج السلوكية تشير إلى أن المعالجة بمستخلص التبغ قد أحدث تغيرات في بعض العناصر السلوكية مثل عدد المربعات المقطوعة ، عدد مرات الجثوم ، زمن الحركة وزمن السكون وزمن التعلق .

النتائج الحيوية تشير إلى أن هناك تغيرات في نشاط أنزيم الاسيتايل كولين أستريز في المخ حيث وجد أنه في الجرعات الصغيرة زاد نشاطه وبينما قل نشاطه في الجرعات العالية .

النتائج عموماً توحي بأن هناك علاقة محتملة بين التغيرات التي حدثت في السلوك الحركي وبين نشاط أنزيم الاسيتايل كولين أستريز في المخ بعد المعالجة بمستخلص التبغ .