

Full Length Research Paper

Spasmogenic, Spasmolytic and Chemical Screening of Cigarettes

Naveed Ullah* and Mir Azam Khan

Department of Pharmacy, University of Malakand, Chakdara, Pakistan.

Accepted 18 June, 2013

The aqueous and ethanolic extracts derived from cigarettes (Morven Gold) were screened for chemicals, spasmogenic and spasmolytic activities. Aqueous extract showed strong relaxant activity that is, 92% against KCl induced contractions while ethanolic extract was found to be moderately spasmolytic (70%). Ethanolic extract was also found to have a strong spasmogenic activity, while aqueous extract depress the spasmogenic activity of pilocarpine induced contractions. Thus, the ethanolic extract was found to be more efficient for spasmogenic activity while aqueous extract was noted to be more efficient for spasmolytic activity. The chemicals found in sufficient quantity in both the extracts were saponin and glycosides. It was also noted that tannins were present only in ethanolic extract in excess quantity. The research indicated clearly that cigarette is a good spasmolytic agent while the ethanolic extract has spasmogenic activity. Further studies are necessary to elucidate its exact mechanism of action.

Key words: Spasmogenic, spasmolytic, chemical screening, cigarette.

INTRODUCTION

Cigarette is a type of smoking tobacco produced and used all over the world. It is a small roll of paper mostly about 7 cm of length and 5 to 7mm of width, filled with a finely, pieced tobacco leaves and may have some additives. At one end of the cigarette, a filter is attached mostly made up of cellulose acetate and of about 2 cm of length. Presently, the use of tobacco is the leading cause of death worldwide (Brundtland, 2000) and is estimated that by 2030 it would be over 10 million annual deaths globally (Warnakulasuriya et al., 2005; John, 2005), 70% of which will be in the developing world (WHO, 2000). It has also been reported that all forms of tobacco carry serious health consequences, most importantly is oral and pharyngeal cancers (Gupta and Ray, 2003; Mack,

2001; IARC, 1985; Merchant et al., 2000; Avon, 2004). In Pakistan, oral cancer is the second most common cancer in women and third most common in men (Jafarey and Zaidi, 1987). Smoking (in form of cigarette) of tobacco has also been reported as a well-known cause of oral squamous cell carcinoma (Gupta et al., 1982; Jayant and Deo, 1986; Brennan et al., 1995; Choi and Kahyo, 1991; Negri et al., 1993). Smoking during pregnancy may cause low birth weight, pre-mature birth and infant death (U.S. Dept. of Health and Human Services, 2001), and also increase the neonatal health care costs (Adams et al., 2002). Furthermore, cigarettes contain carcinogens that not only stimulate genetic damage, but also result in the production of atypical cells, mutations and eventually

cancer, they also impair the function of the p53 gene which, when functioning normally, prevent mutations from developing into cancer (Langdon and Partridge, 1992). It has also been reported that cigarette smoke contains carcinogens that alter biochemical defense systems that lead to deleterious effects on the respiratory tract, heart, pancreas, reproductive tract and other organs (Ostergaard, 1977), and also has a link to common causes of death and disability in elderly aged persons associated with chronic illnesses (Bratzler et al., 2002). But smoking has also been observed to reduce the incidence of various diseases for example, endometrial cancer, and ulcerative colitis, hypertension in pregnancy, Alzheimer's disease and Parkinson disease (English et al., 1995; Graves et al., 1991; Van Duijn and Hofman, 1991). Several other epidemiological studies have also found a beneficial effect of smoking in Parkinson disease (Fratiglioni and Wang, 2000; Checkoway et al., 2002).

In the current study, we present the spasmogenic, spasmolytic and chemical screening of aqueous and ethanolic extract of cigarette. This study was designed with a view to confirm and explore the pharmacological activity of cigarette which contains tobacco and may other ingredients and is used by a large number of people throughout the world.

MATERIALS AND METHODS

Sample material

Two packs of Morven Gold containing 40 cigarettes were purchased from the local market of Abbottabad, Pakistan. A sample pack, marked with a number 1327 was deposited in the Pharmacy Museum, University of Malakand Pakistan.

Preparation of extracts

The materials were withdrawn from each cigarette and were pulverized into fine powder and weighed 13.300 g in duplicate. Each was then extracted in distilled water of 60 ml and ethanol (70%) of 60 ml separately for about 3 weeks. Both the extracts were separately filtered and evaporated under reduced pressure to yield a gum (1.5 to 02 gaqueous and 1.5 to 02 gethanolic) by using Rotary Evaporator and Freez Dryer.

Drugs and standards

Analytical grade chemicals were used in the bioassay technique and chemical screening. All the solutions were freshly prepared in distilled water on the same day of experiments.

Animals and data recording

Rabbits of either sex were bred locally. Their average weight was in the range of 1.5 to 2.0 kg. They were maintained at the "Animal House of Frontier Medical College Abbottabad" as per Byelaws of Scientific Procedures. Animals were given free access to standard diet along with fresh water. Before the start of experiments, animals were given only water and were kept fasted overnight. Intestinal responses were recorded using Organ bath and kymograph.

Spasmogenic activity

The extracts were screened for possible cholinomimetic and spasmolytic activities as per procedure mentioned. Tyrode's solution was prepared having the following concentration (mM): KCl, 2.68; NaCl, 136.9; MgCl₂, 1.05; NaHCO₃, 11.90; NaH₂PO₄, 0.42; CaCl₂, 1.8 and glucose 5.55. The animals were then slaughtered and their abdomens were opened. Rabbit's jejunum portion(s), of about 1.5 to 2 cm length, was isolated and mounted in the tissue bath containing 10 ml of Tyrode's solution maintained at 37°C and supplied with carbogen gas (5% carbon dioxide and oxygen mixture). These portion(s) were kept in Tyrode's solution previously aerated with the carbogen gas (Qayum, 2004). Earlier, the tissues were stabilized for normal activity for a period of about 25 to 40 min. For possible pharmacological screening on the tissues through series of experiments, aqueous and ethanolic extracts of cigarette were tried at doses of 2 and 5 mg/ml. All the doses were applied in cumulative manner and the results were recorded (Farre et al., 1991). The spasmogenic and spasmolytic activity was recorded as given in Figure 1.

Spasmolytic activity

We used the procedure described by Farre et al. (1991) to screen spasmolytic activity. Contractions in the intestine portions were produced by high KCl (80 mM) to depolarize the intestine portions (Farre et al., 1991). The extracts were then applied in similar fashion to relax the tissues and percent relaxation response on KCl induced contractions were recorded as given in Table 1 and shown in Figure 1. The following formula was used for calculations:

$$\% \text{ Inhibition/stimulation} = 100 - \frac{\text{Average height of contractions after extract (mm)}}{\text{Average height of normal contraction (mm)}} \times 100$$

Chemical screening

The aqueous and ethanolic extracts of cigarette were evaluated for the presence of alkaloides, glycosides, terpenes, saponins, tannins, flavonoids and carbohydrates using simple qualitative methods of Sofowora (1993) and Evans (1998). Also the pH of both extracts was recorded.

RESULTS AND DISCUSSION

According to Figure 1, moderate spasmogenic activity of ethanolic extract of cigarette was noted while aqueous extract showed a depressant activity against Pilocarpine induced contractions. By this, it had been confirmed that the cholinomimetic activity of ethanolic extract of cigarette, may be because of the presence of nicotine which may act on any mechanism as discussed. According to Gillespie and Mackenna (1960), the response to nicotine of intestinal preparations *in vitro* is usually a contraction due to stimulation of the parasympathetic cholinergic neurons in Auerbach's plexus. Same was the result recorded in the current study for cigarette. Also, the nature of the medium had been studied which was found to be acidic in both the aqueous and ethanolic extracts.

In another series of experiments, tissues were depolarized with high potassium level (80 mM bath concentration) that produced a sustained contraction (Farre et al., 1991). The test samples were then tried in cumulative

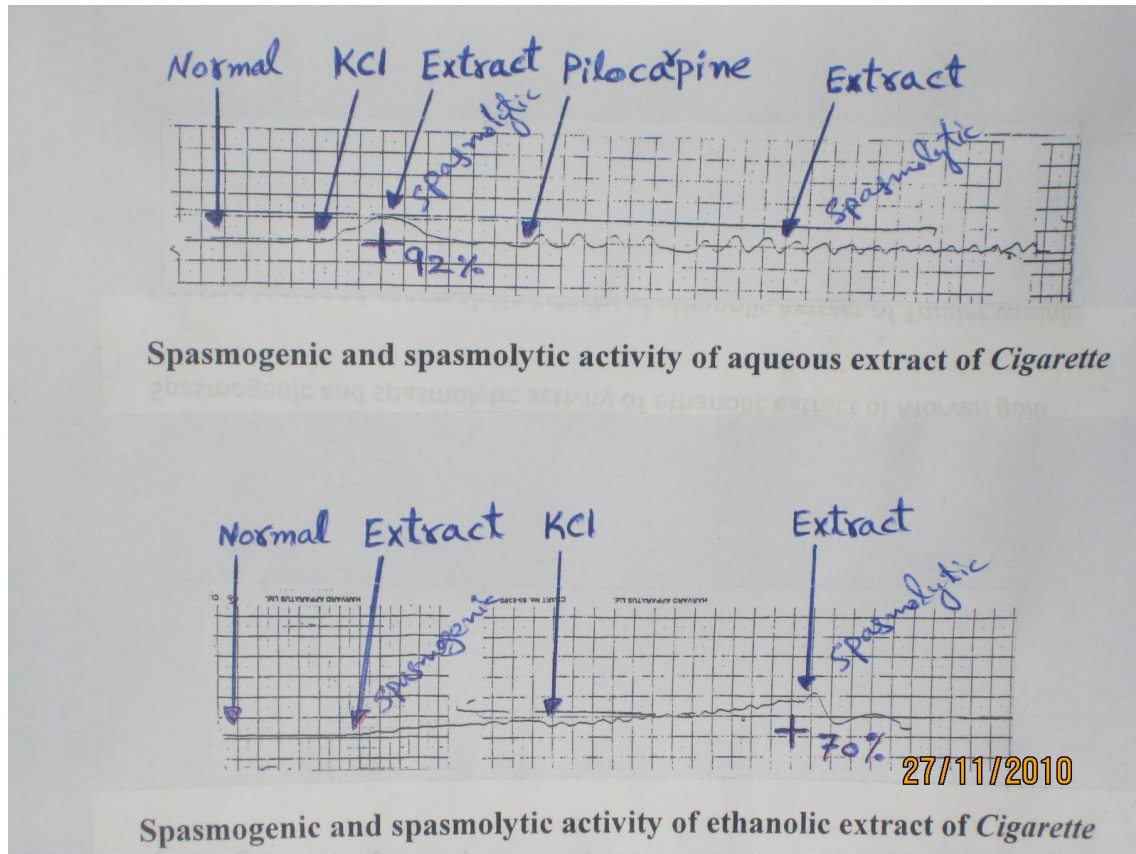


Figure 1. Spasmogenic and spasmolytic activity of crude extracts of cigarette. In the first portion of the graph, aqueous extract of cigarette showed a strong spasmolytic activity, while in the next portion, ethanolic extract showed significant spasmogenic and spasmolytic activity.

manner to observe the spasmolytic effect on the tissues. As it has been postulated that contractions produced by potassium are mediated through calcium channels via influx of calcium from extra cellular fluid and a substance which will inhibit the contraction produced by KCl is considered to have calcium channel blockade (Bolton, 1979). Hence, the extract produced a dose-dependent spasmolytic response on the KCl-induced contractions and is considered to have calcium channel blocking activity. According to Figure 1, the extract produced a spasmolytic effect on the KCl depolarized tissues in dose dependent manner; with a maximum dose of 5.0 mg/ml, KCl-induced contractions (80 mM) were relaxed by the extract in the similar doses. As in the current study, the aqueous extract of cigarette was found to have strong spasmolytic effect, noted as 92%, and for ethanolic extract, it was measured as 70% as given in Table 1 and Figure 1. Positive relaxing effects on KCl induced contraction are mostly referred to calcium channel blocking activity (Gilani et al., 2005). Hence, the spasmolytic activity of cigarette may be mediated through calcium channel blocking activity. On another point of view, Ambache (1946) and Feldberg (1951) obtained evidence that barium could excite ganglionic cells in the intestine for spasmogenic

effect and Ambache (1949) showed that barium excited ganglionic cells. Douglas et al. (1961) reported that the spasmogenic effect of barium in the intestine or superior cervical ganglion were depressed by hexamethonium or nicotine. So from these above discussed mechanisms, may be one would be responsible for the spasmolytic effect of cigarette. Further studies are necessary to elucidate the exact mechanism of action for spasmolytic and spasmogenic activity of cigarette.

The qualitative chemical screening of cigarette revealed the presence of alkaloids and carbohydrates in minute quantity, saponins and glycosides in moderate amount in both the aqueous and ethanolic extracts, while tannins were found only in ethanolic extracts in large amount as given in Table 2.

Conclusion

In the current study, a moderate spasmogenic activity of ethanolic extract of cigarette was found. It was also noted that both extract have a relaxant activity against KCl induced intestinal contraction, which may be due to any of the above discussed mechanism. The spasmolytic activity of the aqueous extract was found to be more effi-

Table 1. Spasmogenic and spasmolytic activity of crude extracts of cigarettes.

Extraction medium	Spasmogenic activity	Spasmolytic activity (%)	Nature
Ethanol	Moderate	70	Acidic
Distilled water	Negative	92 %	Acidic

Aqueous extract were found to be more effective for spasmolytic activity and ineffective in the case of spasmogenic activity while the ethanolic extract were noted to be moderately effective both for spasmogenic and spasmolytic effects.

Table 2. Chemical screening of aqueous and ethanolic extracts of cigarettes.

Test	Observation	Inference	
		Et-Ext	Aq-Ext
Alkaloids :Extract + 10 % tannic acid solution	Turbidity/precipitation	+	+
Saponins: Extract vigorously shaken in a test tube for 2 minutes	Frothing less than 1 cm	++	++
Flavonoids: (Shinoda test) Ethanolic extract + magnesium fillings + conc HCl	Pink or red color	-	-
Tannins: Extract + Few drops of FeCl ₃	An immediate green precipitate formed	+++	-
Terpenes: Decolorized Extract residue + Chloroform + acetic anhydride+conc H ₂ SO ₄	Brown precipitate formed	-	-
Carbohydrates: Extract + Molisch's reagent + conc H ₂ SO ₄	Purple precipitate	+	+
Glycosides: Extract + Fehlings reagent and boild for 2 min	Brick red color	++	++

+, Mild; ++, moderate; +++, excess; -, absent.

cient then the spasmolytic activity caused by ethanolic extract against KCl induced contraction. Further studies are necessary to elucidate its proper mechanism of action. The results also showed the acidic nature of the extracts and the presence of alkaloids, carbohydrates, saponins and glycosides in high and low concentration as given in Table 2.

ACKNOWLEDGEMENTS

This work was sponsored by HEC through Indigenous 5000 Scholarship batch-4. Higher Education Commission, Government of Pakistan is acknowledged for its financial support. We are deeply indebted to the Principal, Frontier Medical College for their Laboratory support and all other participants of this study who gave us their time, especially Ibrar Hussain and Muhammad Zahid Laboratory Assistants, Frontier Medical College Abbottabad Pakistan.

REFERENCES

- Adams EK, Miller VP, Ernst C, Nishimura BK, Melvin C, Merritt R (2002). Neonatal Health care costs related to smoking during pregnancy. *Health Econ.* 11:193-206.
- Ambache N, (1946). Interaction of drugs and the effect of cooling on the isolated mammalian intestine. *J. Physiol* 104:266-287.
- Ambache N, (1949). The nicotinic action of substances supposed to be purely smooth-muscle stimulating. (B) Effect of BaCl₂ and pilocarpine on the superior cervical ganglion *J. Physiol.* 110:164-172.

- Avon SL, (2004). Oral Mucosal Lesions Associated with Use of Quid. *J. Can. Dent. Assoc.* 70:244-8.
- Bolton TB, (1979). Mechanism of action of transmitters and other substances on smooth muscles. *Physiol.* 59:606-718.
- Bratzler DW, Oenhlert WH, Austell A (2002). Smoking in the elderly- it's never too late to quit. *J Okla State Med Assoc* 95: 185-91.
- Brundtland GH, (2000). Achieving worldwide tobacco control. *JAMA* 284:750-751.
- Checkoway H, Powers K, Smith-Weller T, Franklin GM, Longstreth WTJr, Swanson PD (2002). Parkinson's disease risks associated with cigarette smoking, alcohol consumption and caffeine intake. *Am J Epidemiol* 155: 732-38.
- Choi SY, and Kahyo H (1991). Effect of cigarette smoking and alcohol consumption in the aetiology of cancer of the oral cavity, pharynx and larynx. *Int. J. Epidemiol* 20:878-885.
- Douglas WW, Lywood DW, and Straub RW (1961). The stimulant effect of Barium on the release of Acetylcholine from the Superior cervical ganglion. *J. Physiol* 156:515-522.
- English DR, Holman CDJ, Milne E, Winter MG, Hulse GK, Codde JP, Bower CI, Corti B, de Klerk N, Knuiaman MW, Kurinczuk JJ, Lewin GF, Ryan GA (1995). The quantification of drug caused morbidity and mortality in Australia, 1995 edition. Canberra: Commonwealth Department of Human Services and Health.
- Evans WC (1998). *Trease and Evans Pharmacognose 14th Edition* W.B. Saunders Company Limited. 315-316.
- Farre AJ, Columbo M, Fort M, Gutierrez B (1991). Differential effects of various Ca ++ antagonists, *Gen. Pharmacol* 22:177-181.
- Feldberg W (1951). Effects of ganglion blocking substances on the small intestine. *J. Physiol.* 112:177-196.
- Fratiglioni L, Wang HX (2000). Smoking and Parkinson's and Alzheimer's disease: Review of the epidemiological studies. *Behav Brain Res.* 113:117-20.
- Gilani AH, Bukhari IA, Khan RA, Arif-ullah K, Farman U, Viqar UA (2005). Cholinomimetic and Calcium Channel Blocking Activities of *Carthamus oxycantha*. *Phytother. Res.* 19:679-683.
- Gillespie JS, and Mackenna bR (1960). The inhibitory action of nicotine on the rabbit colon. *J. Physiol* 152:191-205.

- Graves AB, Van Duijn CM, Chandra V, Fratiglioni L, Heyman A, John AF, Kokomen E, Konodo K, Mortimer JA, Recca WA (1991). Alcohol and tobacco consumption as risk factors for Alzheimer's disease: a collaborative re-analysis of case-control studies. *Int J Epidemiol* 20(Suppl2):S48-S57
- Gupta PC, Pindborg JJ, Mehta FS (1982). Comparison of carcinogenicity of betel quid with and without tobacco: *An epidemiological review*. *Ecol. Dis.* 1: 213-219.
- Gupta PC, Ray CS (2003). Smokeless tobacco and health in India and South Asia. *Respirology* 8: 419-431.
- IARC (1985). Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Tobacco habits other than smoking: betel-quid and areca-nut chewing and some related nitrosamines. International Agency for Research on Cancer Lyon 37: 141-200.
- Jafarey NA, and Zaidi SH (1987). Cancer in Pakistan. *J. Pakist. Med. Ass* 37:178-183.
- Jayant K, and Deo MG (1986). Oral cancer and cultural practices in relation to betel quid and tobacco chewing and smoking. *Cancer Detect. Prev.* 9:207-213.
- John RM, (2005). Tobacco consumption patterns and its health implications in india, *Health policy* 71:213-22.
- Langdon JD and Partridge M (1992). Expression of the tumour-suppressor gene p53 in oral cancer. *Brit. J. oral maxillofac. Surg* 30: 214-220.
- Mack TM (2001). The new pan-Asian paan problem. *Lancet.* 357:1638-9.
- Merchant A, Husain SS, Hosain (2000). Paan without tobacco: An independent risk factor for oral cancer. *Int J Cancer* 86:128-31.
- Negri E, La Vecchia C, Franceschi S, Tavani A (1993). Attributable risk for oral cancer in northern Italy. *Cancer Epidemiol Biomarkers Prev* 2:189-193.
- Qayum A (2004). Isolated preparations. Guidelines and instructions. In: *Fundamentals of Experimental Pharmacology*, (1st.edn.) New Awan Printers: Peshawar 4:01-4.16.
- Ostergaard K (1977). The concentration and cadmium in renal tissue from smokers and nonsmokers. *Acta Med Scand* 202:193-5.
- Sofowora E A (1993). Phytochemical Assays. In "Medicinal Plants and Traditional Medicine in Africa". 3rd Edition, Spectrum Books Limited Nigeria 150-153.
- U.S Department of Health and Human Services. 2001. Women and Smoking: A report of the Surgeon General.
- Van Duijn CM, Hofman A (1991). Relation between nicotine intake and Alzheimer's disease. *Br Med. J.* 302:1491-94.
- Warnakulasuriya S, Sutherland G, Scully C (2005). Tobacco, oral cancer, and treatment of dependence. *Oral Oncol* 41:244-60.
- World Health Organization (2000). Addressing the Worldwide Tobacco Epidemic through Effective Evidence-Based Treatment. Expert Meeting March 1999, Rochester, Minnesota, USA. In Tobacco Free Initiative.