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Full Length Research Paper

A comparison study of *Morven gold* and *Tander virginia* cigarettes with respect to its spasmogenic, spasmolytic and chemical screening

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The ethanolic extracts derived from cigarettes (*Morven gold* and *Tander virginia*) were screened for chemicals, spasmogenic and spasmolytic activities. *M. gold* extract showed a strong relaxant activity that is 70% against KCl induced contractions while *T. virginia* was found to have a mild spasmolytic activity of (06%). Furthermore, a moderate spasmogenic effect of *M. gold* had being measured, while no measurable spasmogenic activity has been shown by the *T. virginia.* It can be concluded from the current study that *Morven gold* has a strong spamsmogenic and spasmolytic activity, while the *Tander* is not found to be so efficient in either case. The chemicals found in sufficient quantity in both the extracts were tannins, saponin and glycosides. Minute quantity of carbohydrates were also been noted in *M. gold* . The presence of alkaloids were also been noted in excess quantity in *T. virginia* and less amount in *M. gold*. Further studies are necessary to elucidate its exact mechanism of action.

Key words: Spasmogenic, spasmolytic, chemical screening, *Morven gold, Tander virginia.*

INTRODUCTION

Cigarette is a type of smoking tobacco produced and used in 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 some additives. With one end of the cigarette a filter is attached, mostly made up of cellulose acetate and of about 2 cm of length. *Morven gold* and *Tander virginia* are the types cigarette*, M. gold* which is prepared in Pakistan by Lakson Tobacco Company Limited while *T. virginia* is prepared by imperial cigarette industries (Pvt) Ltd. *M. gold* is believed to be a very good quality cigarette and is expensive while the *T. virginia* is believed to be so strong which is not so easy for every one to tolerate and it is so cheaper then *M. gold*. 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 a well- known cause of oral sqamous 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). Further more, cigarettes contain carcinogens that

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not only stimulate genetic damage, but also result the production of atypical cells, mutations and eventually cancer, they also impair the function of the p53 gene which, when functioning normally, prevents 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 tendometrial cancer, ulcerative colitis, hypertension in pregnancy, Alzheimer's disease and Parkinson disease (English et al., 1995; Graves et al., 1991; Van Duijin 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 a comparison between *M. gold* and *T. virginia* with respect to its spasmogenic, spasmolytic and chemical screening. This study was designed with a view to confirm and explore the pharmacological and chemical screening of two types of cigarettes and a comparison between the two, which contains tobacco and other ingredients of different quality.

MATERIALS AND METHODS

Sample material

M. gold and *T. virginia* cigarettes purchased from local market Abbottabad Pakistan. Sample pack, marked with a number 1378 and 1379 has been deposited in Pharmacy Museum, University of Malakand Pakistan.

Preparation of extracts

The materials were withdrawn from each type of cigarette and were pulverized into fine powder and weighed 15 g for either case. Each was then extracted in 70% ethanol. Both the extracts were separately filtered and evaporated under reduced pressure to yield a gum (1.5 to 02 g for each) by using rotary evaporator.

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 breed 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, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 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, ethanolic extracts of *M. gold* and *T. virginia* were tried at doses of 02 and 05 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 the similar fashion to relax the tissues and percentage relaxation response on KCl induced contractions was recorded as given in Table 1, and shown in Figure 1. The following formula was used for calculations:

%Inhibition/stimulation =

$$100 - \frac{Averageheight\ of\ contractions\ after\ extract(mm)}{Averageheight\ of\ normal\ contraction\ (mm)} \times 100$$

Chemical screening

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

RESULTS AND DISCUSSION

Moderate spasmogenic activity of ethanolic extract of *M. gold* had been noted while *T. virginia* extract did not show any spasmogenic effect as shown in Figure 1. By this it can be concluded that, the cholinomimetic activity of ethanolic extract of *Morven*, 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 results were recorded in current study for

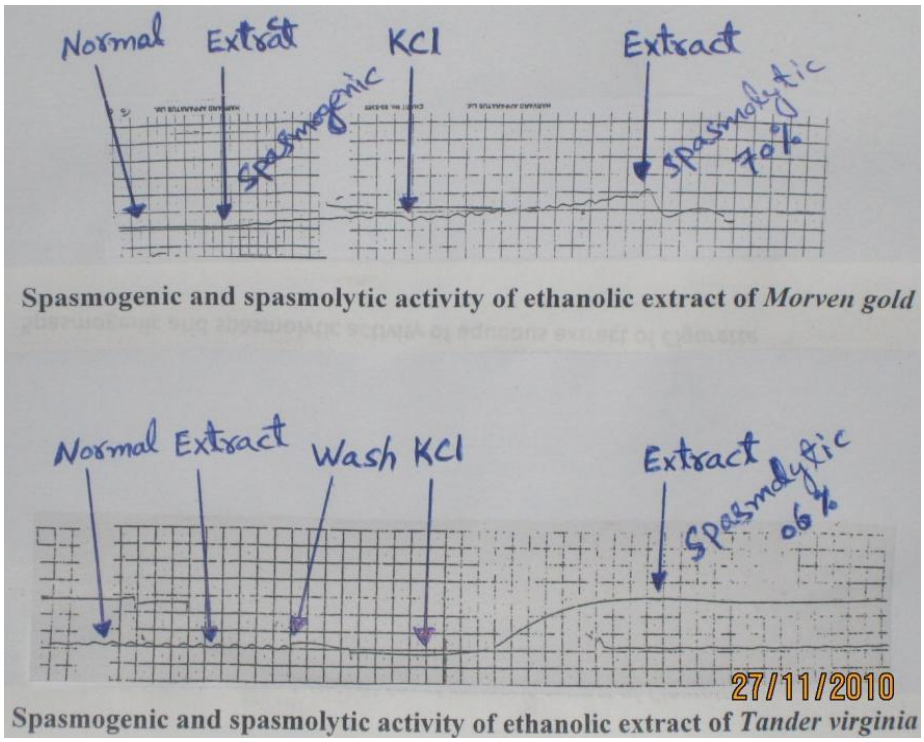

Figure 1. Spasmogenic and spasmolytic activity of *M.gold* and *T. viginia* In the first portion of the graph, *M. gold* showed a good spasmogenic and spasmolytic activity, while in the second portion *T. virginia* showed a mild spasmolytic activity and did not show any significant spasmogenic activity.

Table 1. Spasmogenic and spasmolytic activity of ethanolic extract of *M. gold* and *T. virginia* cigarettes.

Extract	Spasmogenic activity	Spasmolytic activity (%)	Nature
M. gold	Moderate	70	Acidic
T. virginia	Negative	06	Acidic

M. gold was found to be more effective both for spasmogenic and spasmolytic activity, while the *T. virginia* was noted to be a moderate effectiveness for spasmolytic activity.

Morven. Also, the nature of the medium had been studied, which was found to be acidic in both the extracts.

In another series of experiments, the 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 manner to observe the spasmolytic effect on the tissues. As it has been postulated that contractions produced by potassium are mediated through calcium channels through 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). According to Figure 1, both the extracts produced a spasmolytic effect on the KCl depolarized tissues in dose dependant 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 ethanolic extract of *Morven* was found to have strong spasmolytic effect, noted as 70%, and for *Tander* it was measured as 06% given in Table 1, and shown in 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 extracts may be mediated through calcium channel blocking activity. On other point of view, Ambache (1946) and Felberg (1951) obtained evidence that barium could excite ganglion cells in the intestine for spasmogenic effect and Ambache (1949) showed that barium excited ganglion cells. Douglas et al. (1961) reported that the spasmogenic effect of barium in the intestine or superior cervical ganglion was depressed by hexamethonium or nicotine. So, from these aforementioned

Table 2. Chemical screening of *M. gold* and *T. virginia* cigarettes.

Test	Observation	Inferences	
		***Morven* Ext**	***Tander* Ext**
Alkaloids :Extract + 10 % tannic acid solution	Turbidity/precipitation	+	+++
Saponin: 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_2SO_4	Brown precipitate formed	-	-
Carbohydrates: Extract + Molisch's reagent + conc. H_2SO_4	Purple precipitate	+	-
Glycosides: Extract + Fehlings reagent & boiled for 2 min	Brick red color	++	++

+ Mild (present), ++ moderate (present), +++ excess (present), -absent.

discussed mechanisms, may be one would be responsible for the spasmolytic effect of these extracts. Further studies are necessary to elucidate its exact mechanism of action. The qualitative chemical screening of cigarette revealed the presence of tannins, saponin and glycosides. Alkaloids were found in trace amount in *Tander*, while in less quantity in *Morven*. A minute quantity of carbohydrates has also been noted in case of *Morven* as given in Table 2.

Conclusion

In the current study a moderate spasmogenic activity of ethanolic extract of *M. gold* cigarette have been found. It was also noted that both the extract have a relaxant activity against KCl induced intestinal contraction, which may be due to any of above discussed mechanism. The spasmolytic activity of the ethanolic extract of *Morven* was found to be more efficient then the spasmolytic activity caused by ethanolic extract of *Tander* 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, tannins, saponin and glycosides, as given in Table 2.

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