# Behavioural effects of Benylin-Codein in mice

Tijani AY[1], Salawu OA, [1] John–Africa LB[1], Sadiq Abubakar[1] and Chindo BA[1]

[1]Department of Pharmacology and Toxicology National Institute for Pharmaceutical Research and Development
Idu industrial Area Abuja, Nigeria
E-mail: tijaniyahaya2009@gmail.com
Telephone: +234(080) 7215-1058

**Abstract:** The over-the-counter Benylin cough syrup containing codeine (B-C) has emerged as a new agent widely abused in Nigeria among the youths for its opium-like effects such as sedation, euphoria and ability to enhance tolerance for hard work. In the present study the behavioural effects of the cough syrup on locomotion using open field apparatus and short-term memory using Y-maze were evaluated in mice. The behavioural effects of B-C on locomotion and memory were evaluated in mice grouped into four groups of six mice. Groups I served as the control and received 10 ml normal saline/kg body weight while groups II, III and IV received 10.95, 21.90 and 43.80 mg/kg repeatedly for 7-days. Single oral administration of the cough syrup significantly ($p$<0.001) and dose-dependently increased total locomotive activity and rearing in open field. It significantly decreased short term memory indicated by decrease in spontaneous alternation behaviour and increased the total spontaneous motor activity of mice in Y-maze. Repeated administration of the cough syrup significantly ($p$<0.001) and dose-dependently decreased total locomotive activity and rearing in open field apparatus and short term memory in Y-maze. These findings suggest that single and repeated administration of the cough syrup decreased locomotion, rearing and impaired short term memory resulting in sedation, impaired motor and mental activities.
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**Key words**: motor behavior, short-term memory, Y-maze, open field.

## 1. Introduction

Drug abuse is an enormous societal problem due to individual suffering as well as associated disruption of families and communities through criminality and violence, lost productivity, and healthcare costs (More and Li, 1998). Drugs have been abused by mankind for centuries. In the modern era, newer drugs are being sought for the sedation and euphoria they produced which could ultimately results in addiction. The addiction of drugs previously perceived as harmless has been documented all over the world. Codeine is one of such drugs. Abuses of over-the-counter (OTC) cough suppressants have been reported from Japan (Ishigooka et al, 1991) and abuse of codeine separated from over the- counter drugs containing acetyl salicylic acid and codeine have been reported from Denmark (Jensen & Hansen, 1993). Codeine is reported to be associated with higher numbers of reported drug misuse related deaths in the USA (Davis et al, 1991) and is among the top four abused prescription drugs in the elderly population in Washington (Jinks and Raschko, 1990). Fatalities associated with acute overdose of codeine (Bender et al, 1988), as well as neuropsychiatric disorders such as compulsive disorders, anxiety and insomnia were reported by Senjo, (1989) and Ishigooka et al, (1991). Codeine abuse is said to sustain addiction or increase the risk of relapse in patients addicted to other drugs (Stock, 1991). Despite the wide spread misuse of Benylin-codein cough syrup among the teenagers and young adults in Nigeria, there is a paucity of documented reports on this societal problem. Therefore, the present study was designed to evaluate the behavioral effects of Benylin containing codein cough syrup on locomotor activity and spatial memory in Swiss albino mice using open field apparatus and the Y-maze respectively.

## 2. Materials and Methods

### 2.1. Drugs

Commercial Benylin® with Codein was purchased locally from a chemist in Abuja, Nigeria.

### 2.2. Animals

Swiss albino mice (18-20g) of both sexes obtained from Animal Facility Centre of National Institute for Pharmaceutical Research and Development, Abuja Nigeria were used in the study. The Mice had access to feed and water *ad libitum* and maintained under laboratory conditions of temperature (22 ± 1°C), relative humidity (14 ± 1%) and 12 h light and 12 h dark cycle. Experiments were carried out between 9:00 and 15:00 h and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

**2.3. Pharmacological Study:**

**2.3.1. Effect of single and repeated administration**

Swiss albino mice were randomly assigned to control and treatment groups. The control group received 10 ml normal saline/kg body weight while the remaining groups received 10.95, 21.90 and 43.80 mg Benylin-codein /kg body weight orally respectively repeatedly for 7-days. The effect of single administration on locomotion and rearing was evaluated using the open field apparatus while its effect on spontaneous alternation behavior (SAB) in mice was evaluated using the Y-maze one hour after the treatment. The treatment groups for the study of repeated administration effect were the same as that of single administration except that the treatment lasted for 7 days. Evaluation of effects of repeated administration on locomotion and rearing in open field apparatus and SAB in Y-maze were carried out 24 hr after the last dose.

**2.3.2. Open Field Test**

The Open Field (OF) test was used for estimation of spontaneous locomotor (horizontal) and exploratory (vertical) activity (Kalueff et al. 2006; Lalonde and Strazielle (2008). The OF apparatus consist of a clear glass box (45×45 x 40cm). The floor was divided by lines drawn into 9 equally sized squares. One hour after drug administration, each mouse was placed individually in the center of the apparatus. Horizontal (number of squares crossed) and vertical (number of rearing) activity was recorded manually for 5 min. The apparatus was thoroughly cleaned between tests with a tissue paper moistened with 70% methanol.

**2.3.3. Spontaneous alternation behavior in the Y-maze test**

Y-maze test is based on the innate curiosity of rodents to explore novel environments (Luszczki et al., 2005). It has been effectively used to assess exploratory behaviours, learning and memory function in rodents (Kokkinidis et al., 1976; Hahn et al., 1986; Jing et al., 2008; Kwon et al., 2009). The Y-maze apparatus was made of wood with three arms (40 cm long and 6 cm wide with walls 10 cm high) extending from a central platform at 120°. Each mouse was placed at the end of one arm and allowed to move freely through the maze during a session lasting 5 min. Arm entry was defined as the entry of 4 paws into one arm. The maze arms were cleaned with 70% ethanol between tasks to remove residual odours. An actual alternation was defined as entries into all three arms consecutively (that is, ABC, CAB or BCA but not BAB). The percentage alternation for each mouse was determined as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2), multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations) / (Total arm entries-2)] x 100 (Kim et al., 2007; Heo et al., 2009). The total number of arm entries served as an indicator of locomotor activity.

**2.4. Statistical Analysis**

All data were expressed as the mean ±standard error of mean (SEM). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Any significant difference between means was assessed by student's t-test at 95% level of significance.

**3. Results**

**3.1. Effect of single administration on locomotion in open field apparatus (OF)**

At 10.95 mg Benylin/kg body weight, significant ($p<0.05$) increase in horizontal crossing, total locomotor activities and rearing were observed. Significant ($p<0.01$-$0.001$) decrease in horizontal crossing, total locomotor activities and rearing were seen at 21.90 and 43.80 mg/kg body weight when compared to the control respectively as shown in figures 3, 4 and 5.

**3.2. Effect of single administration on spontaneous alternation behaviour**

Significant ($p<0.05$) dose –dependent decrease in spontaneous alternation behavior of mice was observed in the Y-maze test (Fig 1). Significant ($p<0.05$) dose-dependent increase in the spontaneous locomotor activity of mice indicated by increase in the total arm entry was observed (Fig 2).

**3.3 Effect of repeated administration of Benylin-codein on locomotion in open field (OF)**

Significant ($p<0.001$) dose-dependent decrease in horizontal crossings, total locomotive activity and rearing was observed (Fig 8, 9 and10).

**3.4 Effect of repeated administration on spontaneous alternation behaviour**

Significant ($p<0.05$) dose- dependent decrease in spatial memory indicated by decrease in spontaneous alternation behaviour of treated mice when compared to the control was observed. Repeated administration of Benylin–codein significantly ($p<0.05$) decrease spontaneous locomotion as indicated by decrease in total arm entry (fig 6 and 7).

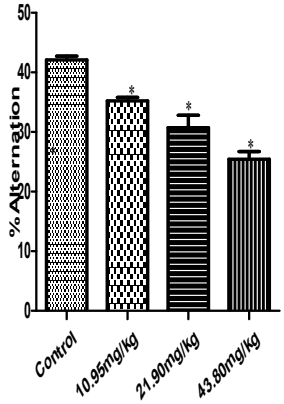


Fig 1: Acute effect of Benylin-codein on Spontaneous alternation behaviour

**Fig. 1a.** Accute effect of Benylin-codein on Spontaneous alternation behavior. The cough syrup produced significant ($p<0.05$) dose-dependent decrease in spontaneous alternation behaviour in mice in Y-maze task when compared to the control. This is significantly different from the control at $p<0.05$, n=6.

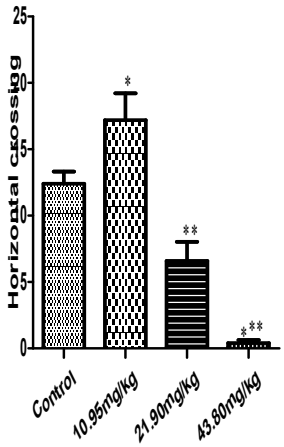


Fig 1:Effect of single admnistration of Benylin-codein on horizontal crossings

**Fig. 1b.** Effect of Single Administration of Benylin-codein on horizontal crossings

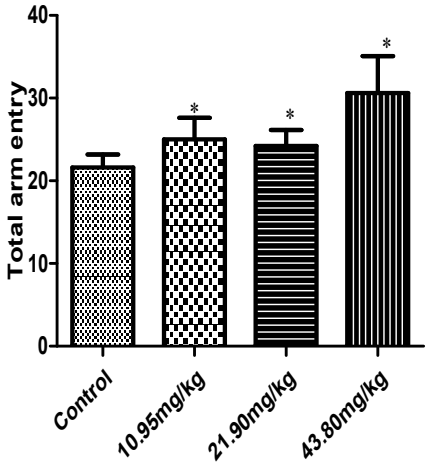


fig 2: Acute effect of Benylin-codein on total arm entry

**Fig. 2a.** Acute effect of Benylin-codein on total arm entry

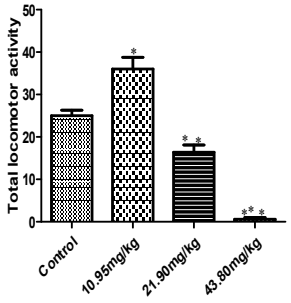


Fig 2: Effect of single administration of Benylin-codein on total locomotive activity(TLA)

**Fig. 2b.** Effect of single administration of Benylin-codein on total locomotive activity (TLA)

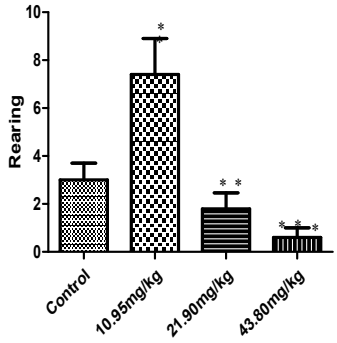


Fig 3: Effect of single administration of Benylin-codein on rearing

**Fig. 3.** Effect of single administration of Benylin-codein on rearing

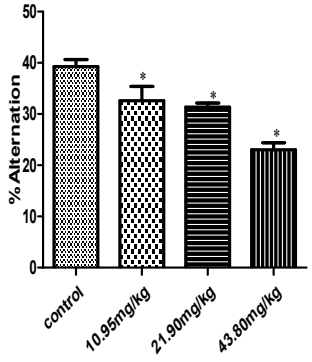


Fig 6: Chronic effect of Benylin-codein on Spontaneous alternation behaviour

**Fig. 6.** Chronic effect of Benylin-codein on Spontaneous alternation behavior

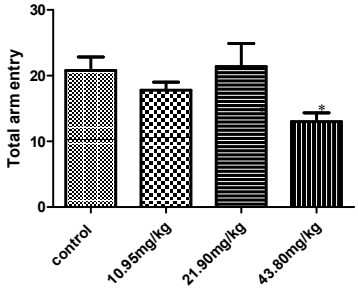


Fig 7: Chronic effect of Benylin-codein on total arm entry

**Fig. 7.** Chronic effect of Benylin-codein on total arm entry

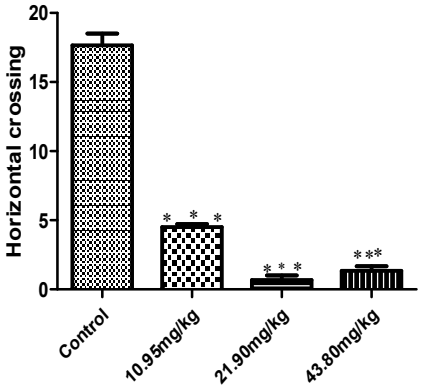


Fig 8: Chronic effect of Benylin-codein on horizontal crossings

**Fig. 8.** Chronic effect of Benylin-codein on horizontal crossings

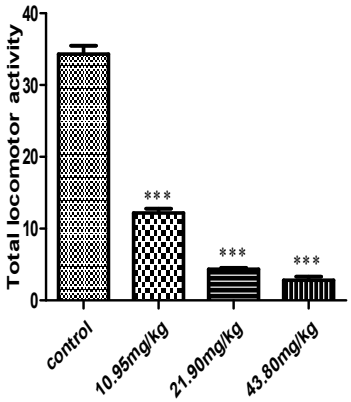


Fig 9: Chronic effect of Benylin-codein on total locomotive activity(TLA)

**Fig. 9.** Chronic effect of Benylin-codein on total locomotive activity (TLA)

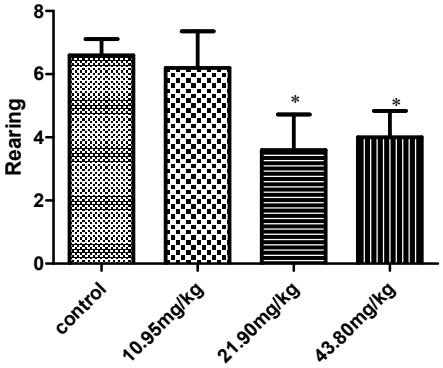


Fig 10: Chronic effect of Benylin-codein on rearing

**Fig. 10.** Chronic effect of Benylin-codein on rearing

## 4. Discussions

The results obtained from this study demonstrated that single and repeated administration of Benylin containing codein cough syrup produced a biphasic effect on open field parameters. An initial hyperactivity characterized by significant increase in locomotion and rearing was observed at 10.95 mg/kg body weight while hypoactivity was observed at doses of 21.90 and 43.80 mg/kg in single administration. However, hypoactivity was observed at all doses used for the repeated administration. Impairment of short term memory characterized by decrease in spontaneous alternation behaviour in mice was produced by both single and repeated administration of the cough syrup at all the doses used.

In the open field test, the number of line crossed and the frequency of rearing are usually used as measures of locomotor activity, exploration and anxiety. A high frequency of these behaviours indicates increased locomotion, exploration and/or a lower level of anxiety. The low dose of 10.95 mg/kg

Benylin cough syrup containing codein produced significant increase in total locomotive activity and rearing in the open field. Interestingly both single and repeated administration of 10.95 mg B-C/kg decreased the spontaneous locomotion in Y-maze as well total locomotive activity and rearing in open field. The initial observation of hyperlocomotion characterized by increased total locomotive activity and rearing is suggestive of reduced anxiety in a novel open field arena. The reduced tensions experienced by naïve users at low dose may soon disappear due to development of tolerance which may prompt gradual increase in the doses used. The development of tolerance to hyperlocomotive effect and increased rearing which are suggestive of anxiolytic (euphoric) effect of B-C disappeared following repeated administration of B-C to mice. The need to sustain the feeling of euphoria characterized by freedom from tension and fear may then lead to development of dependence and ultimately addiction to B-C. Murphy et al, (2001) and Porras et al, (2003) showed that increasing locomotor activity induced by opiates such as morphine and codein in rodents is due to enhanced dopamine release in the striatum. However, development of tolerance to euphoric and sedative effects of repeated administration of the cough syrup may possibly be due to down regulation of dopamine receptors in the striatum. The occurrence of motor disturbance indicated by significant reduction in locomotor activity found among experimental groups in this study further illustrate possible down-regulation of neuronal activity at the striatum .In common with sedatives and hypnotics, small doses of Benylin-codein stimulated behaviour indicated by increased locomotion whereas higher doses produced depressant effect. The depressant effect of the cough syrup containing codein at high doses of 21.90 and 43.80 mg/kg body weight was indicated by the decrease in the rearing and total locomotive activity in the open field study.

Spontaneous alternation behaviour is regarded as a measure of short-term memory in rodents (Hritcu et al, 2007; Heo et al, 2009). A mouse must remember the least recently visited arm in order to alternate the arm choice (Hooper et al, 1996; Lee et al, 2010). The significant decrease in the percentage alternation in the Benylin-codein treated mice on Y-maze showed that single and repeated oral administration of Benylin containing codein cough syrup adversely affected short-term memory processes in mice. Itoh et al, (1994); Schulteis et al, (1988); Stone et al, (1991) and Walker et al, (1991) have all reported that acute administration of opioids impairs cognitive functions in animals. The impairment of spatial memory caused by acute administration of Benylincontainng codein agreed with previous reports which suggested that activation of opioid receptors can decrease the function of cholinergic system and cause memory deficit (Schulteis et al, 1988; Stone et al, 1991). Decker & and McGaugh, (1991) have also demonstrated that opioid agonists such as codein, morphine and β-endorphine, possessing higher affinity for μ-opioid receptors, inhibited cholinergic activity in the hippocampus. Thus, the decrease in cholinergic functions generally caused impairment of the performance in rats' and mice spontaneous alternation tests (McIntyre et al, 2002). The additional constituent of the cough syrup, diphenhydramine, possesses central depressant effects and is particularly prone to cause sedation. It also caused anti-muscarinic effect which may produce synergistic spatial memory impairment with codein present in the cough syrup.

In conclusion, Benylin containing codein cough syrup produced motor dysfunction characterized by reduced total locomotive activity, depressant/ sedative effect characterized by reduced rearing in the open field and impaired short term memory indicated by decreased spontaneous alternation behaviour (a measure of short term memory) of mice in Y-maze.

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**Corresponding author**

Tijani AY
Department of Pharmacology and Toxicology
National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Abuja, Nigeria
E-mail: tijaniyahaya2009@gmail.com
Telephone: +234(080) 7215-1058

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