Journal of Occupational Safety and Environmental Health
A Publication of the Society for Occupational Safety and Environmental Health

EFFECT OF CODEINE CONTAINING COUGH SYRUP ON MOTOR ENDURANCE AND MEMORY IN FEMALE WISTAR RATS

Maryam B. Akor-dewu[1], Maryam S. Abubakar[1], Victor Abah[1], Fatima L. Ciroma, Ahmed-Sherif Isa[1] and Zainab M. Bauchi[2]

[1]*Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Kaduna*
[2]*Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Kaduna*

ABSTRACT
Cough syrups are available in Nigeria as over-the-counter medications which usually contain codeine, with a high potential for abuse. This abuse usually leads to addiction which is now becoming common amongst females. This study investigated the effect of codeine containing cough syrup (Benylin with codeine) on long-term memory and motor endurance in female Wistar rats. The rats were grouped into four groups of five rats each. Group A(control) were given 10ml/kg normal saline while groups B, C and D were administered 10.95 mg/kg, 21.90 mg/kg and 43.80 mg/kg dose of Benylin® with codeine respectively once daily for 2 weeks. Long-term memory and motor endurance were evaluated using the fore paw-grip time test and elevated plus maze respectively. The results showed no significant difference ($p > 0.05$) between the test groups and control for fore paw grip test and elevated plus maze. Based on the findings of this study, it may be suggested that administration of cough syrup with codeine for two weeks did not significantly impair long term memory and motor endurance. Increasing the dose and administration for a longer duration may reveal significant results.

Keywords: Codeine, female rats, motor endurance, long-term memory, cough syrup

INTRODUCTION
Drugs have been abused by mankind for a very long time and have increased in the past two decades (Qiu et al., 2015). Codeine-containing cough syrup is one example of drugs that have been abused for their sedative effects which can subsequently lead to addiction. It has also been reported to be among the top four most abused drugs in Washington (Tijani et al., 2012). Continuous use of codeine has been reported to lead to physical and psychological dependence. Benylin® with codeine is an example of codeine containing cough syrups (Qiu et al., 2015), it contains a number of constituents including diphenhydramine and codeine phosphate. Diphenhydramine and codeine phosphate act together to produce the desired therapeutic effect (Ersek et al., 2003). They are commonly used in preparing cough suppressants, also called antitussives. Antitussives stops the coughing action (Elwood, 1999) by directly inhibiting the medullary cough center of the brain (Burns and Boyer, 2013). Codeine is a derivative of morphine, which is a naturally occurring alkaloid that is gotten from opium and other saps such as Papaver somniferum. Diphenhydramine on the other hand is an antihistamine and sedative agent that is used in the treatment of allergies, cough and nausea, it is an isomer of phenyltoloxamine (Vaikosen et al., 2011). People, who take opioids usually experience a sense of mental dullness (Ersek et al., 2004). They may also assert that they are forgetful, involved in more accidents, have difficulties attending to an activity or task, make mistakes, react slowly, and have difficulties with problem solving, concentration, and thinking (Ersek et al., 2004).

Cognitive function in people taking opioids have been studied and found to cause impairment and poor performances on neural tests. Changes such as hysteria have also been found to occur in patients receiving opioids for pain. Codeine also appears to have a mild and decreased effect on eye-hand coordination and visual acuity (Walker and Zacny, 1998). Codeine also sustains

addiction and increases the risk of relapse in people afflicted with addiction. The side effect and the symptoms appear only after prolonged, chronic use and abuse of the substance and many of these effects are irreversible.

Codeine containing cough syrups are used widely for the treatment of cough, but the abuse of these drugs is becoming extensive. They are mostly abused for their sedative and euphoric properties and more recently; this abuse has been observed in females. Differences in the rate of substance abuse between men and women have been consistently observed in the general population, with males exhibiting significantly higher rates of substance abuse and dependence, but a recent epidemiologic survey suggests that this gap between men and women has narrowed significantly. A survey also found that women engage in the non-medical use of prescription of opioids more often than men (Greenfield et al., 2010). The aim of this work was to study the neurobehavioural effect of codeine containing cough syrup (Benylin® with codeine) on motor endurance and long-term memory using forepaw grip endurance test and the elevated-plus maze test respective in female Wistar rats.

Materials and Methods

Drug

Codeine containing cough syrup (Benylin® with codeine) was purchased from a reputable Pharmacy, Zaria, Nigeria. The constituents of the Benylin® with codeine were diphenhydramine HCl (13.5 mg), codeine phosphate (10.95 mg), ammonium chloride (132.0 mg), sodium citrate (54.4 mg) and menthol (1.1 mg).

Experimental animals

Twenty female Wistar rats weighing an average of 132 g were used for the study. The animals were obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The animals were housed in cages (5 rats per cage) and kept at room temperature and relative humidity. The rats had access to feed and water ad libitum. The rats were also allowed to acclimatize to the laboratory environment before the commencement of the experiment. Experiments were carried out between 8:00 am to 12:00 pm.

Experimental design

The rats were weighed and then randomly assigned into 4 groups of 5 rats each. Rats in group A served as control and were given 10 ml/kg of normal saline, groups B, C and D and were administered Benylin® with codeine orally at a dose of 10.95 mg/kg, 21.90 mg/kg and 43.80 mg/kg respectively for a duration of two weeks. After two weeks, the effect of the drug administration was evaluated using the forepaw grip test to assess for motor endurance and elevated plus maze to assess for spatial long-term memory.

Forepaw grip time test

This forepaw grip test is used as a measure of motor endurance according to the method of Hutter-Saunders et al. (2012). The forepaw grip apparatus consists of two vertical stands with a horizontal pole connecting them. One hour after the last administration, each rat was allowed to grasp the middle pole with its forelimbs and was then gently lowered. The timer was started as soon as the animal was released and the time taken for the animal to release its grasp and fall down to the base was recorded (Van Putten, 2011). The latency to fall was recorded as the index of motor endurance. This test was carried out three times for each animal with a 30 seconds inter-trial period between each test.

Elevated plus maze test (EPM)

Long-term memory was determined using the EPM according to the method of Itoh et al., 1990, which measures spatial memory (Itoh et al., 1990). The maze was composed of wood; it comprised of two open arms (29 x 5cm) and two enclosed (29 x 5 15cm). The maze was supported 15 cm high above the floor.

This procedure exploits preference of a comparatively safe and comfortable environment (the closed arms) to a risky environment (elevated open spaces). The general principle is that the more "impaired" the subjects are due to effect, the less likely they will have retention of memory. The test involves two phases: an acquisition and a test (retention) phase. In the acquisition phase, each rat was placed at the distal end of open arm facing away

from central platform. The transfer latency of the rat to move from the open to the enclosed arms was recorded within 90s. Following entry into the arm, the rats were allowed to explore the apparatus for 30s. Twenty-four hours later, the second phase (retention test) was performed and the rats were observed the time taken for the rat to enter was recorded with maximum time of 90s. After each trial, the maze was wiped with a cotton wool dipped in 70% ethyl alcohol and allowed to dry to remove any olfactory cue or odour. The experiments were conducted in a dimly lit, semi-sound proof room.

Data analysis

Data was expressed as the mean ± standard error of mean (SEM). The analysis was performed using Graph Pad Prism (Version 6.0). Statistical analysis was carried out using Analysis of Variance (ANOVA). Values of p

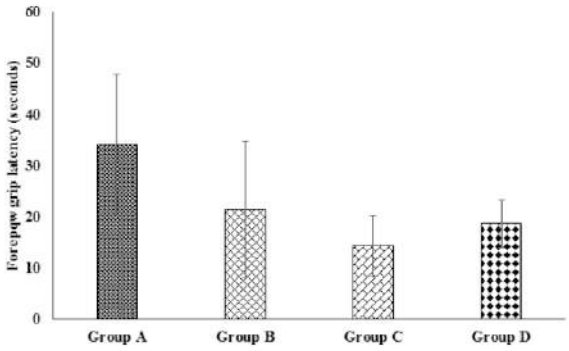

Figure 1: Effect of codeine containing cough syrup on motor endurance in female rats.

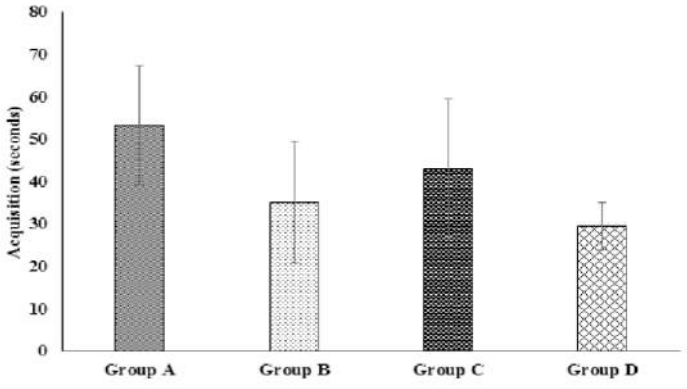

Figure 2: Acquisition latency of female rats administered with codeine containing cough syrup.

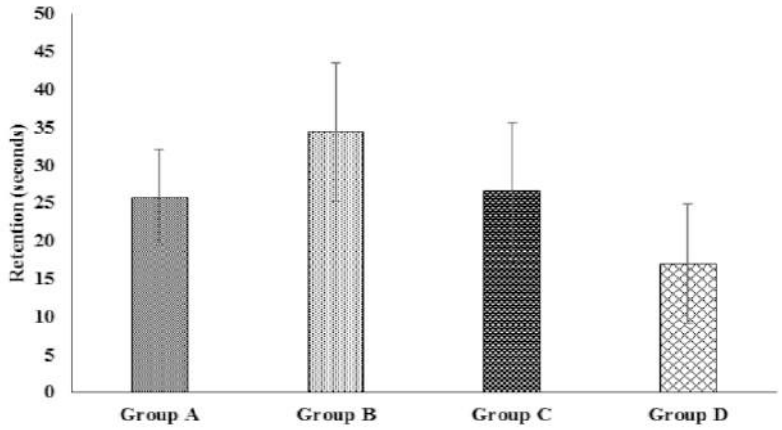

Figure 3: Retention latency of female rats administered with codeine containing cough syrup.

DISCUSSION

The abuse of over-the-counter cough drugs is a continuing problem throughout the world despite media attention and documented dangers. Antitussives exert their clinical effect via relative central inhibition of the medullary cough center of the brain (Qiu *et al*., 2015). Codeine is an opioid agent with cough suppressant activity which occurs via agonism of the opioid receptors (Burns and Boyer, 2013). Codeine is readily absorbed in the gastrointestinal tract and distributed to the liver, spleen and kidney. It crosses the blood brain barrier and is secreted in breast milk (Mulugeta, 2014)

Reduced physical activity observed in animals in this study may be attributed to the administration of codeine containing cough syrup. Tijani *et al.* (2012) demonstrated that repeated administration of codeine containing cough syrup in mice causes a significant decrease in locomotive activity as observed in this study. The reduced physical activity observed in this study could have been from diphenhydramine, a first generation anti histamine, a potent H_1 receptor antagonist and one of the main constituents of cough syrup that causes sedation by readily crossing the blood brain barriers owing to its high lipophilic nature (Kay, 2000). Codeine phosphate, which is also a constituent of the cough syrup acts mostly on m-opioid receptors in the brain and inhibit cholinergic function, which can lead to a decrease in physical activity (Brunton *et al.,* 2010). Thus, the administration of diphenylhydramine and codeine singly have been documented to cause reduction in cognitive and psychomotor performance in addition to sedation (Preston *et al.,* 1992; Kay, 2000).

The result also indicated that repeated administration of codeine containing cough syrup reduced motor endurance using the fore paw grip test. Although, this effect was not statistically significant, the results clearly indicated a reduced motor endurance especially in the group that received 21.90 mg/kg when compared to the control group. The duration of administration of the drug in this study (two weeks) may account for the lack of significance recorded in this study. Furthermore, Van Hout *et al.* (2014) also reported that symptoms appeared only after prolonged chronic use and abuse of the substance, which can sometimes be irreversible.

Cognitive assessment using elevated plus maze did not reveal a statistical significant effect but it showed that the dose of 21.90mg/kg reduced the latency of the rats to find the closed arm. It was also observed that at the dose of 43.80 mg/kg, the latency decreased. The result generally indicates that memory was improved at the highest dose and decreased at the middle dose. Although the reason for this is not fully clear, but a can have different effects depending on the concentration at which it was administered. Essentially, codeine containing cough syrup at the doses and duration administered in this study did not significantly impair motor endurance, and memory. This corroborate previous report that oral codeine and morphine have only modest effects on mood, produce few side effects, and does not impair psychomotor function and performance (Walker and Zacny, 1998). Amato *et al.* (2013) also who reported that codeine/paracetamol did not significantly impair subjective alertness and psychomotor performance. A study conducted to compare brain images of codeine cough syrup addicts and healthy individuals showed changes in the bilateral corpus striatum and decreased weight and volume of the whole brain of the codeine cough syrup addicts. Other long-term effects of codeine use include perforated gastric ulcers, gastrointestinal bleeding, hepatotoxicity, hypokalemia, renal failure and anemia (Frei *et al.,* 2010; Van Hout *et al.,* 2014).

Tijani *et al*. (2012*)* in a study demonstrated that administration of cough syrup impaired locomotion and short-term memory. They found that single oral administration resulted in dose-dependent increases in total locomotive activity and rearing in open field. It is pertinent to state here that there are certain important points worthy of note, which include; the concentration of codeine in the codeine containing cough syrup which may require a longer time to act, the other constituents of cough syrup whether it is diphenhydramine, dextromethorphan or phenylephrine may influence the action of codeine, either by potentiating or inhibition its action.

Considering that the constituent of the codeine containing syrup used in this study was diphenhydramine, Agostini *et al*. (2001) reported that administration of diphenhydramine was associated with a decline in cognitive functions. Although, this was reported in hospitalized elderly patients and diphenhydramine was administered alone. Diphenhydramine has also been reported to show significant reduction in cognitive functions (Gupta *et al*., 2004), and psychomotor performances, but it increases sedation (Preston *et al.,* 1992).

Altogether, codeine may not affect memory because it

acts on opioid receptors unlike other dissociative drugs (ketamine, dextromethorphan) which act via disrupting the actions of the glutamate at N-methyl-D-aspartate (NMDA) receptors on nerve cells throughout the brain whose effects typically depends on the amount of drug taken. The results in this study suggest that the effects of the codeine containing syrup may be progressive as such these effects may not be immediately observed in rats administered the drug for a duration of two weeks. This may be as result of the doses that were administered, the type and species of rats (could be poor metabolizers of codeine) and duration of the study.

CONCLUSION

The results obtained in this study showed that codeine containing cough syrup did not significantly impair motor endurance and memory. It is clear that codeine containing cough syrup has effects on motor endurance, but its effect on memory was not clear. Thus, further studies using various types of learning and memory test model, to determine the effect of codeine containing cough syrup, on learning and memory is imperative. Increasing the dose, and frequency of administration and administration for a longer duration may reveal relevant results in addition to its withdrawal effects.

References

Agostini, J.V., Leo-Summers, L.S. and Inouye, S.K. (2001). Cognitive and other adverse effects of Diphenhydramine use in hospitalized older patients. *Archives of Internal Medicine*, 161, 2091-2097.

Amato, J.N., Marie, S., Lelong-Boulouard, V., Paillet-Loilier, M., Berthelon. C., Coquerel, A., Denise, P., and Bocca, M.L. (2013). Effects of three therapeutic doses of codeine/paracetamol on driving performance, a psychomotor vigilance test, and subjective feelings. Psychopharmacology (Berl), 228(2): 309-320.

Brunton, L.L.; Chabner, B.A. and Knollman, B.C. (2010). Goodman and Gilman's the pharmacological basis of therapeutics (12th Ed). McGraw Hill. pp 481-489

Burns, J. M. and Boyer, E. W. (2013). Antitussives and substance abuse. *Substance Abuse Rehabilitation*, 4:75-82

Daily Trust. (2015) Codeine abuse spikes in women, teens across North [Online] Available from: http://www.dailytrust.com.ng/news/general/codeine-abuse-spikes-in-women-teens-across-north/111459.html#LqW0UpExQ2dUxafr.99. [Accessed: 19th September, 2015].

Elwood, W. N. (1999). Leaning on syrup: The misuse of opioid syrup in Houston. *Texas Commission on Alcohol and Drug Abuse.* 1(1): 5-7

Ersek, M., Cherrier, M. M., Overman, S. S. and Irving, G. A. (2003). The cognitive effects of opioids. *Journal of Pain Management Nursing.* **5**(2): 75-93

Ersek, M., Cherrier, M. M., Overman, S. S. and Irving, G. A. (2004). The cognitive effects of opioids. *Pain Management Nursing*, *5*(2), 75-93.

Frei, M. Y., Nielsen, S., Dobbin, M. D. H. and Tobin, C. L. (2010). Serious morbidity associated with misuse of over-the-counter codeine-Ibuprofen analgesics: a series of 27 cases. *The Medical Journal of Australia.* 193: 294-296.

Greenfield, S. F., Back, S. E., Lawson, K. and Brady, K. T. (2010). Substance abuse in women. *Psychiatric Clinics of North America.* 33 (2): 339-355

Gupta, S., Kapoor, B., Gillani, Z., Kapoor, V. and Gupta, B. M. (2004). Effects of fexofenadine, cetirizine and diphenhydramine on psychomotor performances in adult healthy volunteers. *Journal of Medical Education and Research.* 6 (4):201

Hutchings H.A. and Eccles . R. (1994). The opioid agonist codeine and antagonist naltrexone do not affect voluntary suppression of capsaicin induced cough in healthy subjects. *Eur Respir J,*

7(4):715-9.

Hutter-Saunders, J. A. L., Gendelman, H. E. and Mosley, R. L. (2012). Murine motor and behaviour functional evaluations for acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication. *Journal of Neuroimmune Pharmacology.* **7** (1): 279-288.

Itoh, J., Nabeshima, T. and Kameyama, T. (1990). Utility of an elevated plus maze for the evaluation of memory in mice: effect of nootropics, scopolamine and electroconvulsive shock, *Psychopharmacology,* 101: 27–33.

Kay, G.G. (2000). The effects of antihistamines on cognition and performance. *Journal of Allergy and Clinical Immunology*, 105(6, part 2): s622-s627.

Mulugeta, L. (2014). Pharmacogenetics of codeine. *U. S. Food and Drug Administration,* 1: 2-8.

Preston, K. L., Wolf, B., Guarino, J. J. and Liriffiths, R. R. (1992). Subjective and behavioral effects of diphenhydramine, lorazepam and methocarbamol: evaluation of abuse liability. *Journal of Pharmacology and Experimental Therapeutics.* 262 (2): 707-20

Qiu, Y. W., Su, H. H., Lv, X. F. and Jiang, G. H. (2015). Abnormal White Matter in Chronic Users of Codeine-containing Cough Syrups: A Tract-based Spatial Statistics Study. *American Journal of Neuroradiology.* 36 (1): 50-56

Substance Abuse and Mental Health Services Administration. (2009). Physiological effects of alcohol, drugs and tobacco on women. *Substance Abuse and Mental Health Services Administration.* 51:3

Tijani, A. Y., Salawu, O. A., John-Africa, L. B., Sadiq, A. and Chindo, B. A. (2012). Behavioral Effects of Benylin-Codeine in Mice. *Nature and Science.* 10 (4): 83

Vaikosen, N. E., Benjamin, U. E. and Ebi, A. (2011). Simultaneous Assay of Codeine Phosphate and Diphenhydramine hydrochloride in Cough Mixtures by Zero-Order Derivative UV Spectrophotometry. *African Journal of Pure and Applied Chemistry.* **5** (5):104-110

Van Hout, M. C., Bergin, M., Foley, M., Rich, E., Rapca, A. I., Harris, R. and Norman, I. (2014). A Scoping Review of Codeine Use, Misuse and Dependence, final report. CODEMISUSED Project European Commission 7th Framework Programme, EU. Brussels. pp 10, 89, 90.

Van Putten, M. (2011). The use of hanging wire tests to monitor muscle strength and condition over time. SOP ID number DMD_M.2.1.004. Treat- NMD Neuromuscular Network .pp 3-8

Walker, D. J. and Zacny, J. P. (1998). Subjective, Psychomotor and Analgesic Effects of Oral Codeine and Morphine in Healthy Volunteers. *Psychopharmacology,* 140: 191-201.