Original Research Article

EFFECT OF CHRONIC ORAL EXPOSURE TO OVERDOSE OF COUGH SYRUPS ON RATE OF DNA FRAGMENTATION IN LIVER AND BRAIN OF RATS

ABSTRACT

This study was designed to determine the effect of chronic oral exposure of overdose of cough syrups containing codeine (CSC) and dextromethorphan (DXM) on the DNA fragmentation in the liver and brain of Wistar rats. Forty-five rats divided into 9 groups of 5 rats were used. Groups 1, 2, 3 and 4 were treated with 0.1, 0.2, 0.4 and 0.6 mL/kg b/w of CSC, respectively for 21 days while Groups 5, 6, 7 and 8 were treated with the same doses of DXM, respectively for 21 days. Group 9 (control) received 0.4 mL of distilled water once daily and all the rats were sacrificed 24 hours after the last treatment. DNA analysis was done on the harvested liver and brain. Significant reductions ($p < 0.05$) in the rate of DNA fragmentation of the liver tissues were observed in all the groups treated with the overdose of cough syrups when compared to the control. However, there was no significant difference in the rate of DNA fragmentation of the brain in the all the groups treated with cough syrup as compared to the control. The result indicates that overdose of cough syrup may cause suppressed DNA fragmentation of the liver thereby predisposing the organ to dysfunctions and untimely aging.

KEYWORDS: Cough syrup, overdose, DNA fragmentation, codeine, dextromethorphan

INTRODUCTION

Cough syrup is a medication produced and used for suppressing cold symptoms or cough and it is usually purchased over the counter (Smith et al., 2014). Cough syrups consist of various constituents such as codeine and dextromethorphan (DXM). Codeine is one of the globally available and used opiates often used as an analgesic, antitussive, antidiarrheal agent and a mild to moderate pain reliever (Tremlett et al., 2010; Frost et al., 2012; Derry et al., 2015). It can be solely used to suppress cough or combined with another drug (Carney et al., 2018). It acts by depressing the central pathways of the cough reflex in the brain (Modu & Bugaje, 2017). The long-term use of products containing codeine in combination with ibuprofen or paracetamol could cause conditions like nephrotoxicity, pancreatitis or gastric ulcers (Van Hout, 2014). Codeine is mostly metabolized in the liver and minimally in the central nervous system (CNS) and intestine (Frost et al., 2016). In the brain, codeine is metabolized into morphine. Concomitant administration of codeine affects normal functioning of the liver and despite being unable to cross the blood-brain barrier, codeine still elicits some morphine-related effect which further confirms the metabolism of codeine into morphine (Stingl et al., 2013). Overdose of codeine could have depressive effects on the CNS or death because of respiration arrest. Despite being a weak opiate, there is the potential for abuse and misuse, physical and psychological dependence can also occur as a result of long-term use of codeine containing cough syrups (Nielsen et al., 2015; Wu et al., 2016). The minimal lethal oral dose for codeine is approximately 0.5-1.0g (17-34 pills containing 30mg of codeine) (Baselt 2008). Continuous overdose of products containing codeine can cause gastric ulcers, nephrotoxicity and hepatotoxicity (Nielsen & Van Hout, 2015). Approximately 33 million people use substances containing codeine every year and most people use these substances for the ecstasy it present to those using them (Reeves et al., 2015). According to a study by Modu & Bugaje (2017), the misuse of codeine containing cough syrup is a fast growing trend amongst youth and students between 20-30 years in Nigeria and about 6 million bottles are sold daily

in the northwestern Nigeria among students in primary and secondary schools due to its cheaper price and easy availability (Sadiq & Uba, 2017).

DXM is also highly effective in the suppression of cough and the daily allowable dosage is about 120mg/day (Chyka et al., 2007). Due to its availability, effectiveness and safety at appropriate doses, it is the most widely used cough suppressant. DMX can induce a psychosis characterized by paranoia, hallucination and delusions when consumed at doses higher than 150mg/day (Martinak et al., 2017). It acts on the cough reflex of the medulla oblongata and increases the cough reflex threshold by 50 years (Hu et al., 2020). Therapeutic overdose of DXM has been shown to affect the central nervous system and cause tachycardia, hypertension, ataxia, disorientation, confusion, impaired coordination and hallucinations (Linn et al., 2014; Paul et al., 2017; Karami et al., 2018). Dextrorphan, an active metabolite of DXM is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist and is metabolized by cytochrome P450 (isoenzyme CYP2D6) (Silva & Dinis-Oliveira, 2020). CYP2D6 expression is controlled genetically and the polymorphism of the enzyme can influence the toxicity of a cough syrup containing DXM especially when illicitly combined with other drugs or used at inappropriate dosage (Brown et al., 2018). Due to the misuse and abuse of cough syrup with either codeine or DXM as main ingredient among youths in Nigeria, the production and importation was banned by the Nigerian Government in 2018 (Owoade et al., 2019). The abuse of cough syrups has been discovered to cause damages to the white matter of brain (Qui et al., 2015), folate deficiency (Au et al., 2007), aberrant functional organization and volume loss in the ventral medial prefrontal cortex (Qui et al., 2014).

Studies have investigated the effect of overdose of cough syrup containing codeine and DXM on biochemical indices but there is no documented study on the effect on the rate of DNA fragmentation in the liver and brain. Therefore this study was designed to evaluate the effect of overdose of cough

syrup containing codeine and DXM on the rate of DNA fragmentation on the liver and brain tissues.

MATERIALS AND METHODS

Tutolin containing codeine produced by Tuyil Pharm Industry Limited and Greenlin produced by Greenlife Pharmaceuticals Limited were purchased from a registered pharmacy store. The normal approved dosage of the cough syrup is 10 mL, which is taken four times a day. From this data, any dosage above this quantity can be considered an abuse. A total of 35 Wistar rats were randomly distributed into seven groups.

Group A: Control (0.5 mL of distilled water)

Group B: Cough syrup containing codeine (CSC-1) (0.1 mL)

Group C: Cough syrup containing codeine (CSC-2) (0.2 mL)

Group D: Cough syrup containing codeine (CSC-3) (0.4 mL)

Group E: Cough syrup containing dextromethorphan (DXM-1) (0.1 mL)

Group F: Cough syrup containing dextromethorphan (DXM-2) (0.2 mL)

Group G: Cough syrup containing dextromethorphan (DXM-3) (0.4 mL)

Extraction of DNA from organs

Extraction of DNA from animal tissues was carried out using the Qiagen DNA extraction kit which uses the spin column method. 25mg of tissue (10mg for spleen) was cut into small pieces, placed in a microcentrifuge tube and 180µL of Buffer ATL and 20µL of proteinase K were added. The content was mixed thoroughly on a vortex mixer and incubated at 56^{o}C overnight until the tissues were completely lysed. After complete lysis of the tissue, 200 µL of buffer AL was added, mixed thoroughly followed by the addition of 200µL of ethanol (96 - 100%). The whole mixture (including any precipitate) was pipetted into the DNeasy mini spin column placed in a 2mL collection tube and centrifuged at 6000xg for 1 minute. The flow through was discarded while the spin column was placed

in a new 2mL collection tube. After this, 500µL of buffer AW1 was added and centrifuged at 6000xg for 1 minute. The flow through was discarded while the spin columnwas placed in a new 2mL collection tube and 500µL of buffer AW2 was added, centrifuged at 20000g for 3minutes to dry the DNeasy membrane and flow through was discarded. The DNeasy mini spin column was then placed in a clean 2mL microcentrifuge tube and 200µL of buffer AE was pipetted directly onto the DNeasy membrane and incubationwas carried out at room temperature for 1 minute and then centrifuged at 6000xg for 1minute to elute the DNA. The eluted DNA was quantified using Fisher Scientific Nano drop spectrophotometer and kept at -20^{o}C until used (Ausubel, 2002).

Electrophoresis of extracted DNA

1% agarose gel (100mL) was prepared in 1X TAE (Tris Acetate EDTA) buffer, boiled in a microwave oven to dissolve the agarose, allowed to cool to about 60^{o}C and 5µL of ethidium bromide was added and mixed thoroughly. The agarose gel was then poured into the gel tray after the comb has been fixed in the tray. The agarose was allowed to solidify and the comb was removed to create the wells where the DNA was loaded. Loading buffer (2 µL) was addedto 18µL of DNA extracted. and this mixture was loaded along with DNA marker into the wellsof the gel. After loading, electrophoresis was carried out at 70Volts for 45 minutes in 1X TAE buffer and DNA was viewed using UVP gel documentation system (Alonso, 2012).

RESULTS

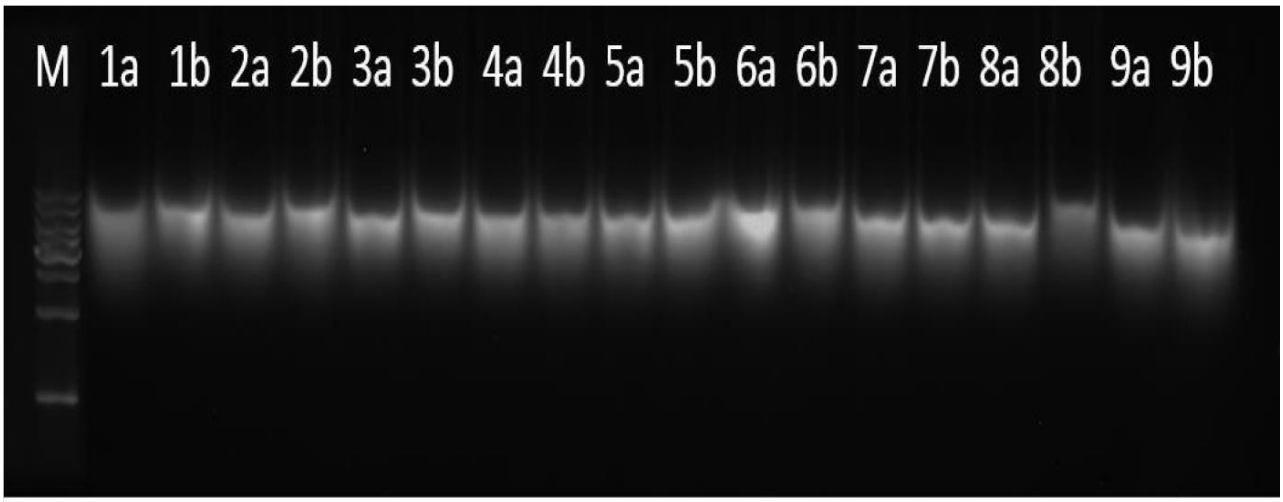

Figure 1: Analysis of Codeine containing cough syrup and DMX containing cough syrup on DNA fragmentation in the liver revealed by agarose gel electrophoresis.

Lane 1a and 1b treated with 0.1mg/kg bd wt of codeine, Lane 2a and 2b treated with 0.2mg/kg bd wt of codeine, Lane3a and 3b treated with 0.4mg/kg bd wt of codeine, Lane 4aand 4b treated with 0.6 mg/kg bd wt of codeine, Lane 4a and 4b treated with 0.1mg/kg bd wt. DXM, Lane 5a and 5b treated with 0.2mg/kg bd weight DXM, Lane 6a and 6b treated with 0.4mg/kg bd wt DXM, Lane 7a and 7b treated with 0.6 mg/kg bd wt DXM, Lane 9a and 9b treated with distilled water.

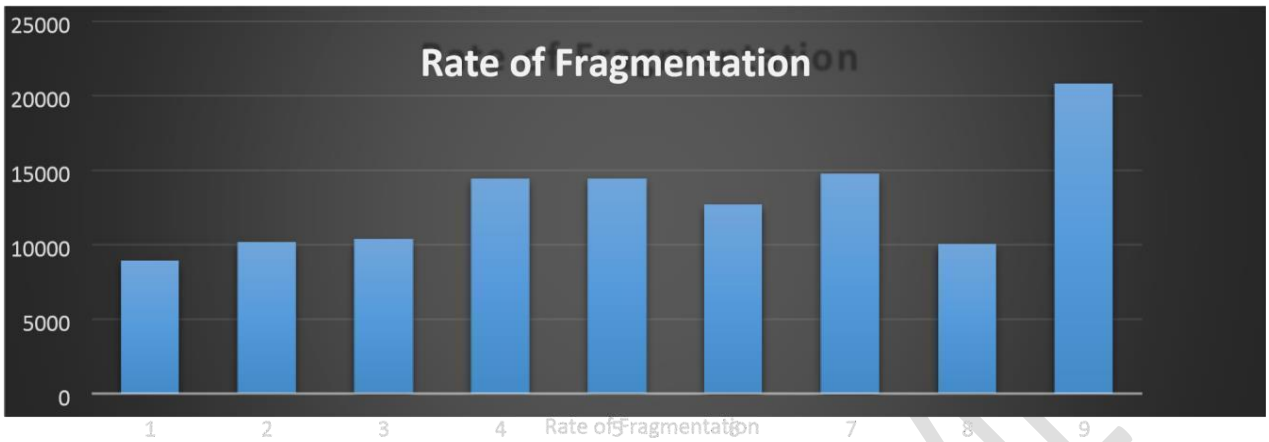

Figure 2: Histogram showing rate of liver DNA fragmentation suppressed by cough syrup containing Codeine and Dextromethorphan

Codeine and Dextromethorphan suppressed genomic DNA fragmentation in Hepatic cell. The two cough syrups influence on DNA fragmentation was evaluated by measuring the level of fragmented DNA by detecting DNA ladders on agarose gel electrophoresis. It was observed that Group1 showed marked suppressed DNA fragmentation (43%) as compared with the control (99%). Analysis of DNA from apoptic cell by agarose gel electrophoresis produced a characteristic DNA pattern, which is regarded as a biochemicalhallmark of apoptosis. The two cough syrups showed a typical DNA ladder pattern in the liver of the rat.

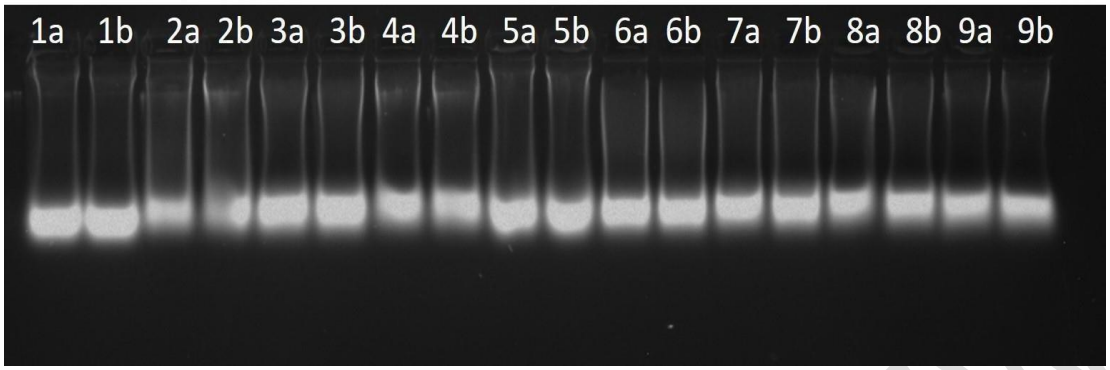

Figure 3: Analysis of DNA fragmentation of brain influence by Codeine containing cough syrup and Dextromethorphan containing cough syrup revealed by agarose gel electrophoresis Lane 1aand 1b treated with 0.1mg/kg bd wt of codeine, Lane 2a and 2b treated with 0.2mg/kg bd wtof codeine, Lane3a and 3b treated with 0.4mg/kg bd wt of codeine, Lane 4aand 4b treatedwith 0.6 mg/kg bd wt of codeine, Lane 4a and 4b treated with 0.1mg/kg bd wt Dextromethorphan, Lane 5a and 5b treated with 0.2mg/kg bd wt Dextromethorphan, Lane6a and 6b treated with 0.4mg/kg bd wt Dextromethorphan, Lane 7a and 7b treated with 0.6mg/kg bd wt Dextromethorphan, Lane 9a and 9b treated with 4.0 distilled water.

DISCUSSION

Cough syrups containing either codeine or DXM are being used to suppress cough or cold symptoms. It causes drowsiness, weakness and dizziness when a normal dosage is used. The long-term usage of cough syrup has been discovered to cause hepatotoxicity, nephrotoxicity and also have depressive effects on the CNS among other side effects. The abuse of cough syrups containing codeine or DXM among the youths has become a global public health concern and is becoming a rapidly growing trend in Nigeria.

Codeine is commonly used to treat mild-to-moderate pain and coughs. It is classified as an opioid

which means it has morphine-like properties and this is because codeine is metabolized into morphine (Stingl et al.,2013) which makes it effective in relieving pain. DMX is an antitussive agent which acts on the cough reflex in the medulla oblongata (Hu et al., 2020). Exposure to an overdose of cough syrup containing DXM causes hallucination, intoxication, paranoia and delusions (Martinak et al., 2017).

In a previous study by Kayode et al., (2021), elevated levels of AST, ALP and ALT were observed in Wistar rats exposed to overdose of cough syrup containing codeine and DXM. An elevation in the levels of these enzymes could be an indicator of liver damage or disease (Hyder et al., 2013), this is because the liver is a site of multiple oxidative reactions with formation of free radicals (Kayode et al., 2021). An elevation in ALT is a precise indicator of liver inflammation and AST also increases due to infiltration of the liver cells as a result of damage of the mitochondria (Mallo et al., 2013). It has been observed that the abuse of DXM products containing acetaminophen could also result in damage to the liver (NIDA, 2017).

DNA fragmentation is the main biochemical feature of apoptosis which is the body's mechanism of eliminating excessive, mutated, damaged and infected cells (Majtnerová & Roušar, 2018). It can also be attributed to permanent cell death, apoptosis or necrosis in the nuclear DNA exposed to oxidative stress (Ismail et al., 2016). ROS-mediated oxidative stress has been discovered to attack DNA and cause DNA lesions such as base modifications, single-strand or double strand breaks, oxidized purines and pyrimidines which could lead to mutations, genomic instability and cell death (Yang et al., 2011).

In this study, significant reductions ($p < 0.05$) in the rate of DNA fragmentation in the liver tissues were observed in all the groups treated with the overdose of cough syrups containing codeine and DMX when compared to the control group. In the codeine containing cough syrup

group, group 1 had the lowest rate of DNA fragmentation while in the DXM containing cough syrup group, group 8 had the lowest rate of DNA fragmentation. The reduced DNA fragmentation rate in the liver tissue observed in this study could pose a problem indicating the suppression of apoptotic process which could lead to the accumulation of excess, mutated and damaged cells. This could prevent the development of new hepatocytes thereby subduing the regenerating ability of the liver.

Normally the liver is a very active organ in terms of cell turnover and cells or tissue regenerations which helps to rejuvenate and restores worn tissues of the liver. Any substance or drug that reduces or suppresses this ability of the liver will result into rapid and untimely aging of the liver. Invariably, such liver may have expired, aged or 'pack up' earlier than normal. These may result in many related liver diseases, reduced physiological and biochemical functioning of the liver. Failure of removal of DNA from damaged cells could also lead to autoimmune diseases (Majtnerová & Roušar, 2018).

In humans and rats, there is the expression of CYP2D6 in the hepatic and brain microsomes (DuBois and Mehvar 2018). CYP2D6 is an enzyme involved in the metabolism of Dextrophan, an active metabolite of DMX and its can influence the lethality of a cough syrup containing DXM especially when used at inappropriate dosage (Brown et al., 2018). This could be a reason for the suppression in the rate of DNA fragmentation in the liver.

In a study by Archibong et al., 2021, decrease in SOD and CAT and increase in MDA levels which is an indicator of oxidative stress was observed in prolonged administration of codeine containing cough syrup. This could also result in glucose hypometabolism eventually leading to brain failure. In this study, there was no significant difference in the rate of DNA fragmentation of the brain in the all the groups treated with cough syrup as compared to the control group. This could be as a result of the inability of the substances to cross the blood-brain barrier.

The expression CYP2D6 in the brain is about 1-4% of the expression in the liver (DuBois and Mehvar 2018). This could be the reason for the insignificance in the rate of DNA fragmentation observed in the brain tissues in this present study.

CONCLUSION

Overdose of codeine and DMX containing cough syrup decreased the rate of DNA fragmentation in the liver but the brain showed no decrease in the rate of DNA fragmentation. This suppression of DNA fragmentation could predispose the organ to dysfunctions and untimely aging.

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