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Histopathological Effect of Emzolyn Codein Cough Syrup on Lungs and Its Oxidative Stress Biomarkers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Despite the dangers associated with the increased use of codeine drugs, limited researches have addressed the specific effects of emzolyn codeine on the lung. The aim of this study was to assess the histological effects of emzolyn codeine cough syrup on the lung of Wistar rats and its oxidative stress. Twenty one (21) Wistar rats were divided into 3 groups labeled T1, T2 and T3. Group T1 served as control and was given distilled water and diet for 42 days, group T2 was treated with 0.1 mg/g bodyweight emzolyn codeine cough syrup for 21 days while group T3 was treated with 0.1 mg/g bodyweight emzolyn codeine cough syrup for 42 days. At the end of the duration, the wistar rats were sacrificed under anaesthesia and the lungs were collected after dissection and transferred into 10% buffered formalin. Sections of the lungs were obtained and processed for histological studies using Hematoxylin and Eosin stain, Periodic acid Schiff's solution, Phosphotungstic acid Haematoxylin stain and Methanamine Silver stains. Results from the study suggested that acute and chronic exposure to emzolyn codeine cough syrup produced significant ($P<0.05$) decrease in body weight, edematous aveolar space with marked type 11 pneumocyte,

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marked hypertrophy (H) of the septa and marked inflammatory cells. The levels of total antioxidant status (TAS) was also determined using standard spectrophotometric techniques. The mean MDA of the exposed groups were significantly higher while the mean levels of SOD, GPx, CAT, and GSH were significantly lower than the control group. In conclusion, this study confirmed the risk of increased oxidative stress, pulmonary toxicity and decreased body weight due to emzolyn codeine cough syrup administration. Thus, indiscriminately and prolong use emzolyn codeine drug should be avoided and antioxidant supplements are advised as a prophylactic supportive therapy for adequate measures in preventing development of oxidative stress-associated complications among exposed individuals.

Keywords: Histopathological effect; emzolyn codein; cough syrup lungs; oxidative stress biomarkers.

1. INTRODUCTION

History of drug use for therapeutic purpose is as old as medical practice itself. Drugs used for therapeutic purposes also in some cases become toxic to the patient. The reason is attributed to the fact that drug is a substance that brings about change in biological function through its chemical actions [1]. Cough is an important protective reflex, contributing significantly to the innate immunity of the respiratory system by enhancing mucociliary clearance. Cough is one of the most frequently managed problems in primary care. The most common causes of acute cough are viral upper respiratory tract infections (URTIs) and acute bronchitis.

There are many types of drugs that are used to suppress cough and are often prescribed in combination. Before dealing with the particular type of drug used, it is important to consider briefly the nature of cough production, its role in disease and desirability of suppressing it [2]. The treatment of an acute cough is directed primarily at decreasing the cough in addition to treating the underlying cause. Symptomatic relief of cough can be provided by over-the-counter or prescription cough remedies. The treatment of a chronic cough will also be directed at treating the underlying condition [3]. It is important to recognize that treatment may be difficult, may employ multiple approaches, and may not completely eliminate the cough. The treatment of a cough will depend largely on its severity and underlying cause [4].

Codeines cough syrup may be used for the relief of coughs associated with the common cold, bronchitis, influenza, and other selected disorders. Codeine therapy is intended to increase the volume and decrease the viscosity of bronchial secretions so that they can be more easily cleared by the mucociliary system and by coughing [5].

Emzolyn codeine cough syrup is a pleasant, raspberry flavored syrup which is indicated in the control of dry, unproductive cough, and in the alleviation of nasal and bronchial congestion. Emzolyn codeine contains Diphenhydramine hydrochloride, which has central sedative, local anesthetic, spasmolytic and anti-cholinergic (diminishes upper respiratory tract secretions) properties, in addition to its main antihistaminic actions [6]. In addition, relatively high ammonium chloride content provides an effective codeine action reducing the viscosity of the mucus, which can then act as a demulcent, thereby protecting inflamed and irritated surfaces and inducing a productive cough which is less exhausting and less painful to the patient. Diphenhydramine hydrochloride has additive or synergistic effects with alcohol and other CNS depressants such as hypnotics, sedatives, tranquilizers, etc. MAO inhibitors prolong and intensify the anticholinergic (drying) effects of antihistamines. The codeine acts by signaling the body to increase the amount or hydration of secretions, resulting in more yet clearer secretions and as a byproduct lubricating the irritated respiratory tract. It inhibiting the cough center in the brain, elevating the threshold for coughing [7]. The actual effectiveness of codeines is highly questionable and poorly researched, and they are sometimes thought to be based on subjective clinical impression and tradition, hence the need of this study.

1.1 Aim of the Study

To determine the histopathological effect of emzolyn codeine cough syrup on lungs of wistar rat and its oxidative parameters

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out in Animal House of Ebonyi State University Abakaliki, Histopathology Department Federal Teaching Hospital Abakaliki.

2.2 Animal Procurement

A total of twenty one (21) wistar rats were purchased from the animal house of Ebonyi State University Abakaliki, Ebonyi State. The wistar rats were weighed and found to weigh between 153g to165g. They were acclimatized and grouped into two namely: control and test groups.

Chart 1. Experimental design

Experimental groups	Treatments given	Duration of treatment
T1 (control) 7 wistar rats	Distilled water and diet	Forty-two (42) days
T2 (Test) 7 wistar rats	0.1 mg of emzolyn per gram weight of the rat and diet twice daily	Twenty-one (21) days
T3 (Test) 7 wistar rats	0.1 mg of emzolyn per gram weight of the rat and diet twice daily	Forty-two (42) days

2.3 Sample Size Estimation/Sample Selection

Resource Equation method (E) was used for the sample size estimation
E= (total number of animals in a group X number of groups) – number of groups as recommended by Jaykara and Kantharia (2003) on how to calculate sample size in animal studies. Therefore, 7 X 3 = 21 – 3 = 18
Sample Size =18.

Corrected sample Size = Sample Size **E**÷ (1-**%attrition**)

In this study, 10% attrition is expected, hence,

Corrected sample Size =18/1-10/100
18/0.9
20

2.4 Inclusion Criteria

Eligibility of the subjects includes; healthy wistar rat, body weight between 150g to 200g and must be male wistar rat.

2.5 Exclusion Criteria

All non-male rats were excluded and also male rats weighing between 150g and 165g were included

2.6 Food Administration

Food (poultry finisher) and water was administered to the control group while food and emzolyn was given to those in the test group. The exercise lasted for six (6) weeks in total.

2.7 Collection of Samples

At the end of the duration, the wistar rats were sacrificed under anaesthesia and the lungs were collected after dissection.

2.8 Treatment of Samples

The tissues collected from the treated and control rats were preserved in fixative awaiting tissue processing.

2.9 Histopathological Studies

Tissue samples of liver and lungs in 10% buffered formalin and were processed for histopathological examination. A routine method of dehydration in ascending series of ethanol, clearing with xylene and embedding in paraffin was used. Sections of 5 µm thickness were sliced using microtome and were stained with Hematoxylin and Eosin stain, Periodic acid Schiff's solution, Phosphotungstic acid Haematoxylin stain and Methanamine Silver stains, mounted and viewed.

2.10 Staining Procedure

2.10.1 Haematoxylin and Eosin (H&E)

The embedded tissue section was deparafinize with xylene for 15mins (two xylene changes) and then taken to alcohol for 5mins (three descending grade). It was then rinsed with water and stained with haematoxylin for 8mins. The tissue `was the blued in scot's tap water for 5mins and rinsed with water. The tissue was differentiated in 1% acid alcohol for 30secs and then stained with eosin for 5min. The tissue was taken to alcohol for 5 mins (three ascending grade). The tissue was cleared in xylene (I & II) for 15mins and mounted with DPx [8].

2.10.2 Phosphotungstic acid Haematoxylin (PTAH)

The embedded tissue section was deparafinize with xylene for 5mins (three xylene changes) and

then taken to alcohol for 5mins (two alcohol changes). It was then oxidize in potassium permanganate for 10 mins and rinsed. The tissue section was transfer in oxalic acid for 5 mins to bleach. PTAH stain was added 24 hrs and then transferred to 95% alcohol then absolute alcohol. The section was dehydrated, cleared and mount with DPx [8].

2.10.3 Periodic acid schiff's solution

The section was deparaffinize in xylene for 5 mins (three xylene changes) and transferred to alcohol for 5 mins (two alcohol changes). Periodic acid was added for 10 mins and properly washed. Schiff's solution was then added for 20mins. The section was counter stained in haematoxylin, blue in tap water, dehydrated, cleared and mounted in DPx [8].

2.10.4 Methanamine silver technique

The section was dewaxed, dipped in alcohol and rinsed with water. 1% periodic Schiff was added for 10mins and rinsed. The section was then transferred to preheated hexamine silver solution. 0.1% gold chloride was added for 2 mins to tone the section. 5% sodium thiosulphate was added for 5mins to fix the section and then counter stained in 0.2% light green for 1min [8].

2.11 Determination of Oxidative Stress Parameters

2.11.1 Determination of catalase (CAT) activity

2.11.1.1 Procedure

Exactly 2.5 ml of phosphate buffer and 2 ml of hydrogen peroxide (H_2O_2) were added to the test tube. After that, 0.5 ml of the sample was also added to the test tube. To 1 ml portion of the reaction mixture, 2 ml of dichromate acetic acid reagent was added. Absorbance was read at 240 nm against the bank at a minute interval.

2.11.1.2 Calculation

$$\text{Catalase concentration } \left(\frac{\text{U}}{\text{L}}\right)$$

$$= \frac{0.23 \times \log \text{Absorbance } 1/\text{Absorbance } 2}{0.00693}$$

2.12 Estimation of Superoxide Dismutase (SOD) Activity

2.12.1 Procedure

Exactly 0.2 ml of the sample was introduced into 2.5 ml of 0.05 phosphate buffer. At pH of 7.8, 0.3 ml of newly prepared adrenaline solution was added to the reaction mixture followed by quick mixing by inversion in the cuvette. The increase in absorbance was taken every 30 seconds for 3 minutes at 480 nm against blank. Blank contained 0.3 ml of adrenaline and 2.5 ml buffer.

Super Oxide Dismatase (SOD) activity was measured by determining the inhibition of auto oxidant of adrenalins.

2.13 Determination of Malondialdehyde (MDA) Level

2.13.1 Procedure

Exactly 0.1 ml of sample, 0.9 ml of distilled H_2O, 0.5 ml of 25% TCA reagent and 0.5 ml of 1% TBA reagent in 0.3% NaOH were added to a test tube. The test tube was incubated at 95^0C for 40 minutes. After that, the test tube was allowed to cool in water and exactly 0.1 ml of 20% SDS (sodium dodecyl sulphate) was added to the test tube. The absorbance of the sample was read against the blank reagent at 532 and 600 nm.

2.13.2 Calculation

$$\%\text{TBARS} = \frac{\text{A532} - \text{A600}}{0.5208 \times 0.1} \times 100$$

2.14 Reduced Glutathione Determination (GSH)

2.14.1 Procedure

One mililitre of the sample was added 4.0 % sulfo-salicyclic acid and the mixture centrifuged at 3,000 rpm for 15 minutes at 2 ^{o}C. The samples were introduced to 4.5 ml of Ellman reagent and absorbance was measured at 412 nm. The blank were prepared by addition of 0.5 ml of 4 % sulfo-salicyclic acid to 4.5 ml of Ellman reagent while absorbance was measured at 412 nm.

Plasma GSH concentration = $\frac{\text{Asample - Ablank}}{\text{Astandard - Ablank}}$ X Cstandard

2.15 Determination of Glutathione Peroxidase Activity

2.15.1 Procedure

The reaction mixtures were prepared by putting in a test tube 14.0 ml of distilled water, 2.0 ml 5 % pyrogallol solution, 1.0 ml of 0.147 M H_2O_2 solution and 2.0 ml of 0.1 M phosphate buffer (pH 6.0). The mixture was then equilibrated at 20 ^{0}C for about 5 minutes, afterwhich there was the addition of 1.0 ml of the sample solution, with mixing of the resulting solution. This was followed by the addition of 1ml of 2.0 N H_2SO_4 to stop the reaction after exactly 20 seconds. The optical density of the resulting solution was measured at 420 nm against a blank (prepared like the test except that no sample is added to it and 15 ml of distilled water is used where 14 is used in the test solution).

2.15.2 Calculation

The activity of peroxide can be calculated for using the formula:

$$\text{Volume of activity } (\mu/\text{ml}) = \frac{\Delta\text{OD x df}}{0.117 \text{ x Vs}}$$

where: $\Delta OD = (OD_{Test} - OD_{Blank})$

= ΔOD x 8.547 x df -Weight activity (µ/mg) = (µ/ml) x $\frac{1}{c}$

$$\frac{\Delta OD\ 8.547 \times df}{c}$$

Where: Vs = sample volume (1.0ml); 0.117 = Optical density at 420 nm corresponding to 1 mg % purpurogallin in ether; df = dilution factor (if used during the study); c = enzyme/sample concentration in dissolution.

2.16 Statistical Analysis

The results were expressed as means ± standard deviation. Data were compared using the one-way analysis of variance (ANOVA). This was followed by students' T-test comparison between the different treated groups, and differences were considered to be statistically significant at $p < 0.05$.

3. RESULTS

3.1 Photomicrograph of Wistar Rat on Oral Emzolyn Codeine Cough Syrup

The photomicrograph reports of the wistar rat on both acute and chronic administration of emzolyn codeine cough syrup has shown degrees of lung damage in both acute and chronic administration as shown in plate 2-3; compared with plate 1 the normal section of the lung without any drug intervention.

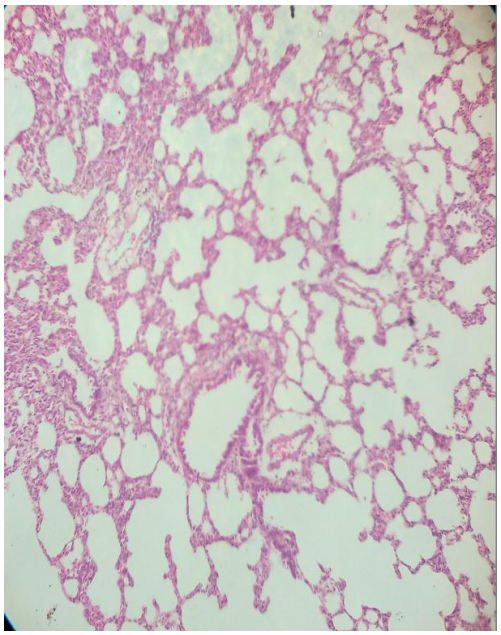

Plate 1a. Mag. X40

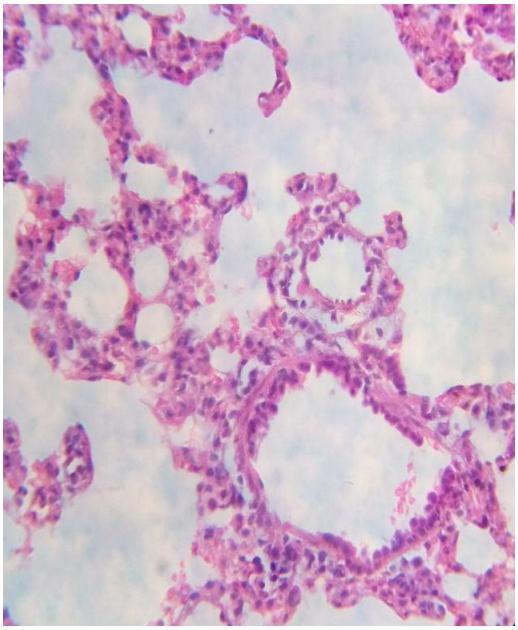

Plate 1b. Mag. X40

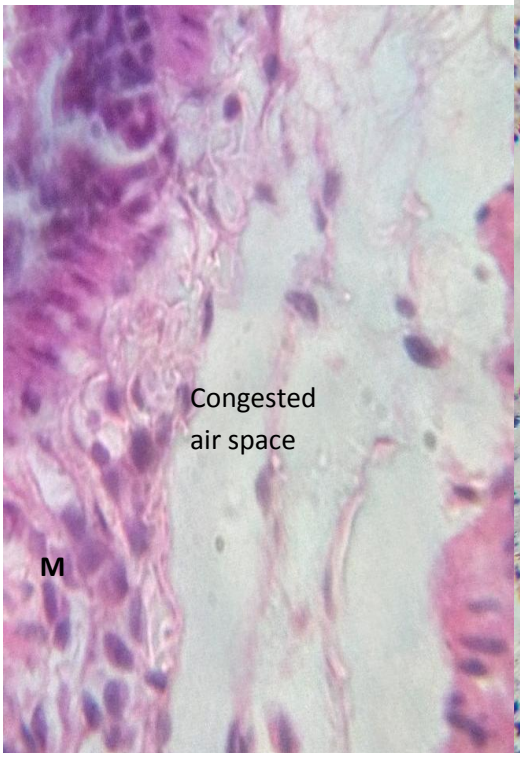

Plate 2a. Mag. X40

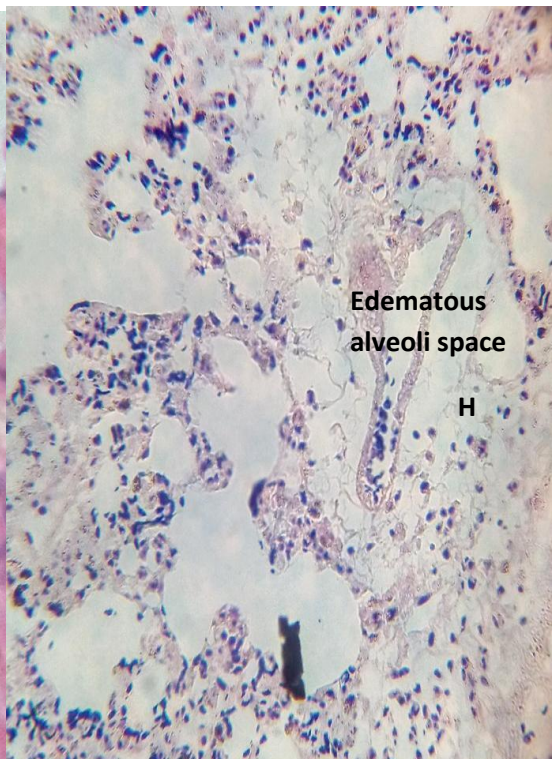

Plate 2b. Mag. X40

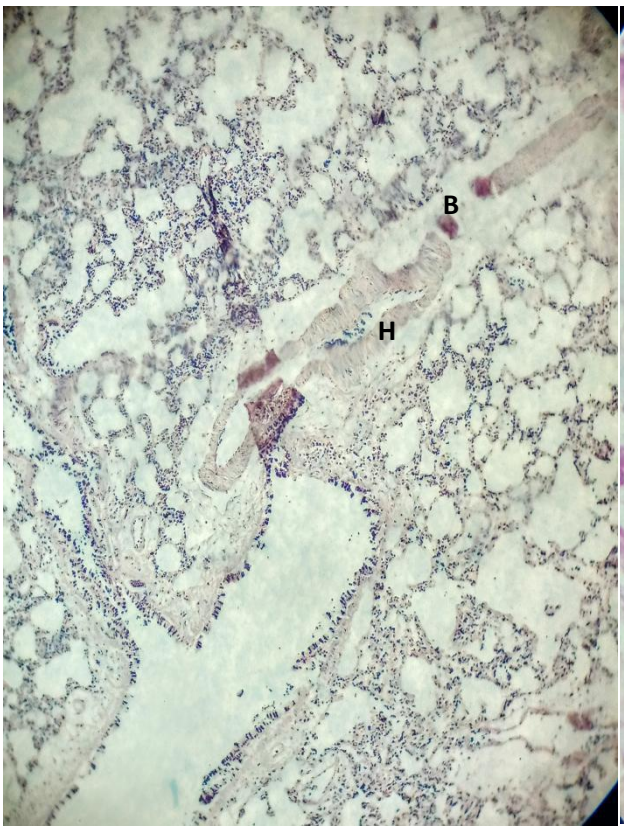

Plate 3a. Mag. X40

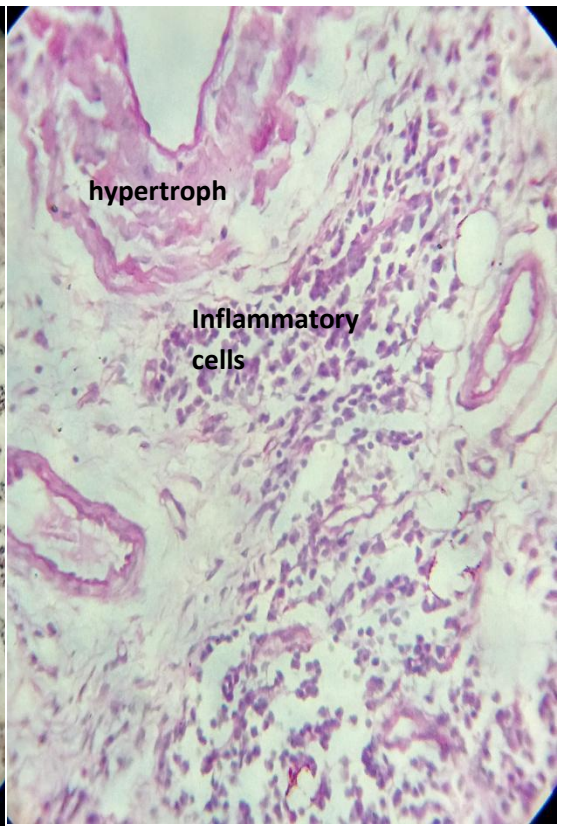

Plate 3b. Mag. X40

Plate 1a and b are lung histology sections of a normal wistar rat stained with Periodic acid Schiff's solution and haematoxylin eosin technique respectively; both sections shown normal air spaces and pleural septa. Type 1 numerous type 1pneumocyte and few type 11 are also seen.

Plate 2a is a section of lung tissue of a wistar rat on acute administration of emzolyn codeine cough syrup, section stained with haematoxylin and eosin staining technique shown congested edematous aveolar space with marked type 11 pneumocyte. Plate 2b a chronic administration of enzolyn codeine cough syrup, section stained with phosphotungstic haematoxylin shown edemateous alveoli space with marked hypertrophy (H) of the septa.

Plate 3a is a section of lung tissue of a wistar rat on acute administration of emzolyn codeine cough syrup, section stained with methanamine silver technique, section shown marked hypertrophy0 of smooth muscles and blood (**B**)as a consequence of lung injury. Plate 3b a chronic administration of enzolyn codeine cough syrup, section stained with Periodic acid Schiff shown edemateous alveoli space with marked hypertrophy (H) of the septa and marked inflammatory cells.

3.2 Effects of Emzolyn Codeine on Lung Oxidative Stress Indices in Normal Rat

Administration of Emzolyn codeine in normal rats significantly ($p<0.05$) reduced the activities of catalase, SOD, GPx, level of GSH and increased the level of MDA as shown in Figures 1-5.

3.3 Effects of Enzolym Codeine on the Body weight in Rat

Administration of Enzolym codeine in rats significantly ($p<0.05$) reduced the body weight of the experimental rats (Fig. 6).

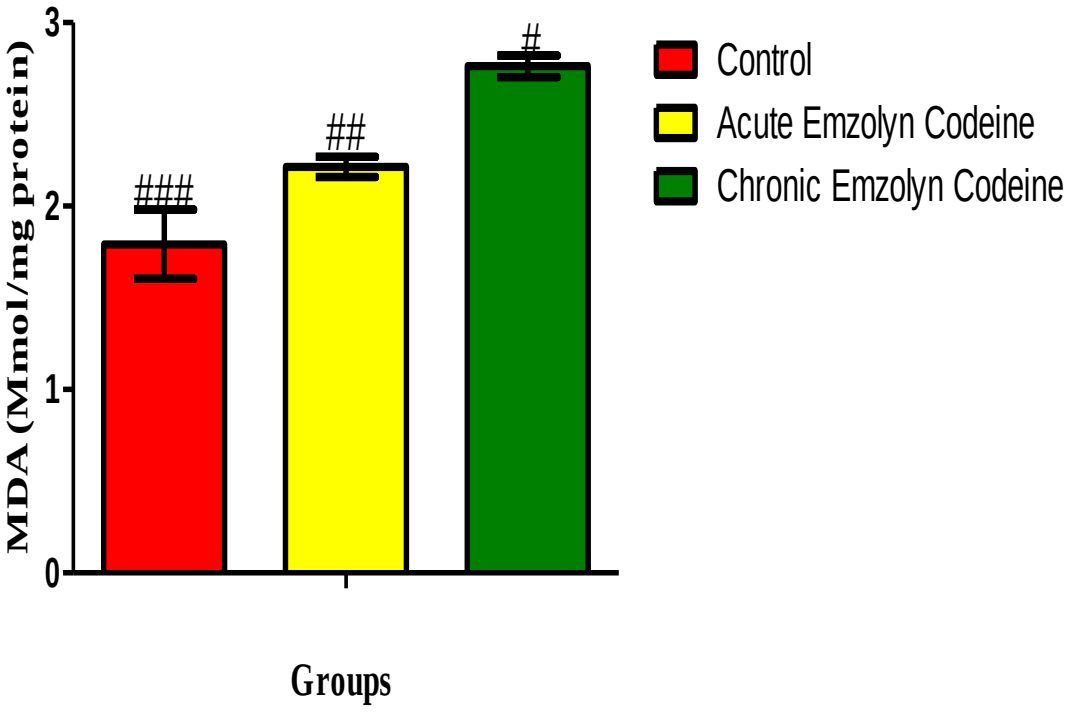

Fig. 1. Effects of Enzolym codeine on MDA Level in Normal Rats. Mean values with different sign are significantly different at P<0.05

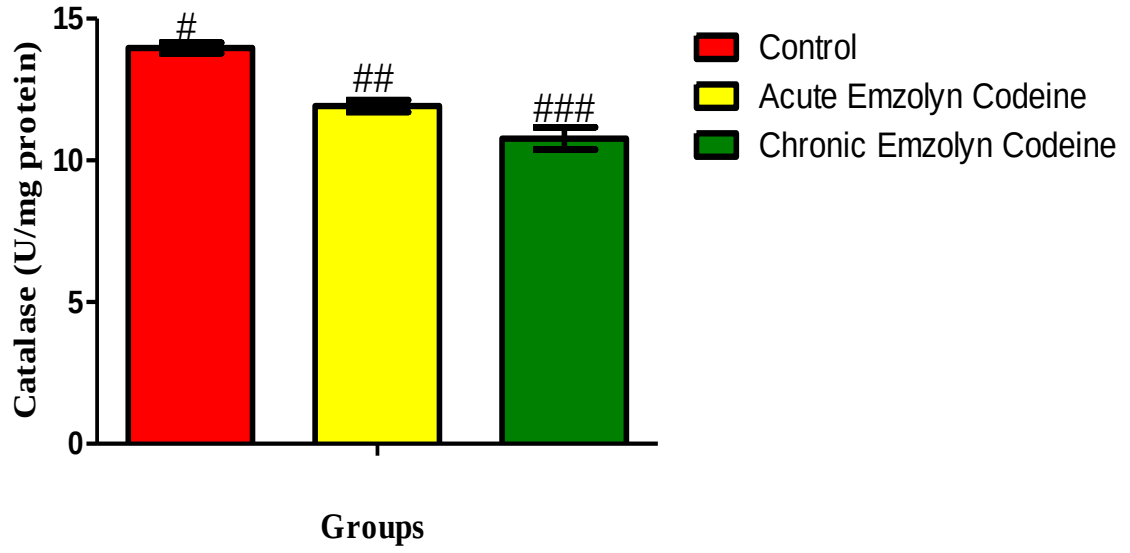

Fig. 2. Effects of Enzolym codeine on Catalase Activity in Normal Rats. Mean values with different sign are significantly different at P<0.05

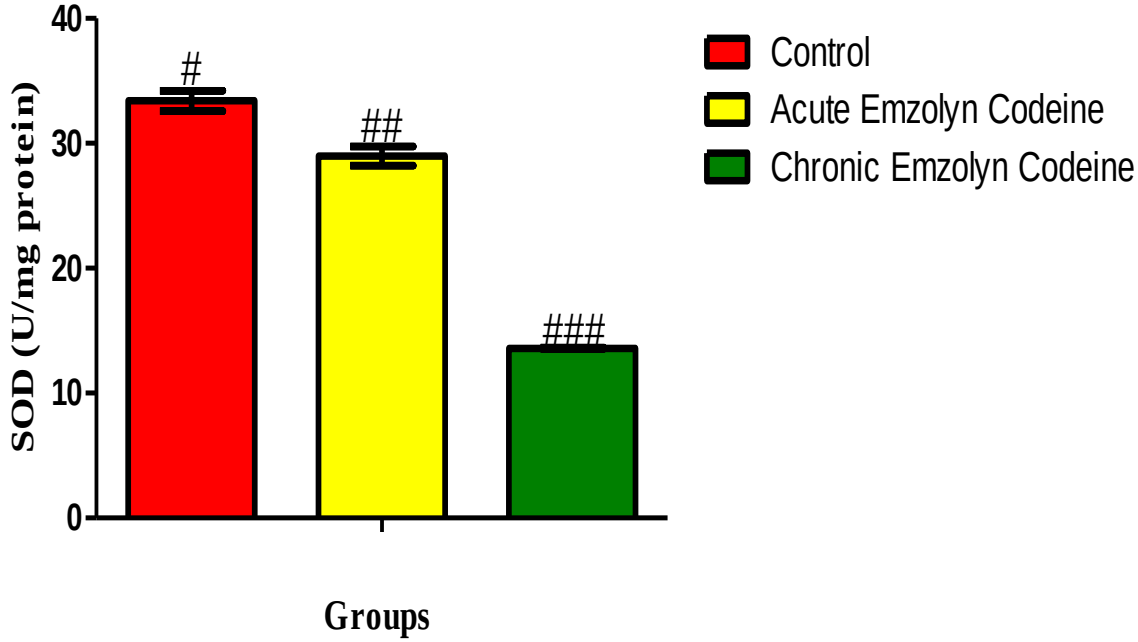

Fig. 3. Effects of Enzolym codeine on SOD Activity in Normal Rats. Mean values with different sign are significantly different at P<0.05

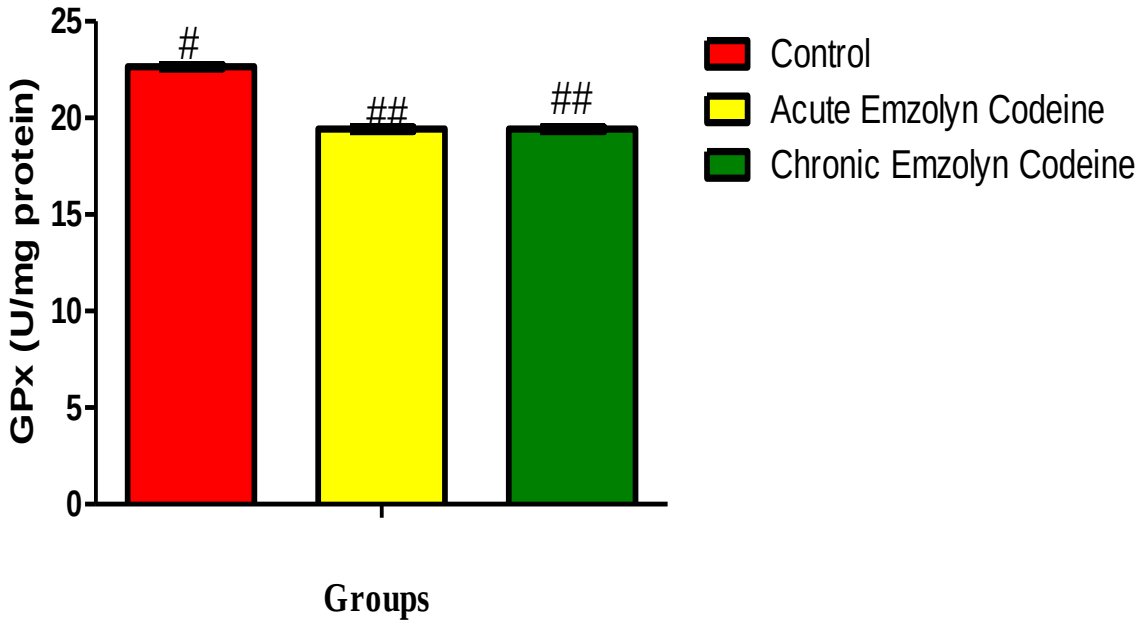

Fig. 4. Effects of Enzolym codeine on GPx Activity in Normal Rats. Mean values with different sign are significantly different at P<0.05

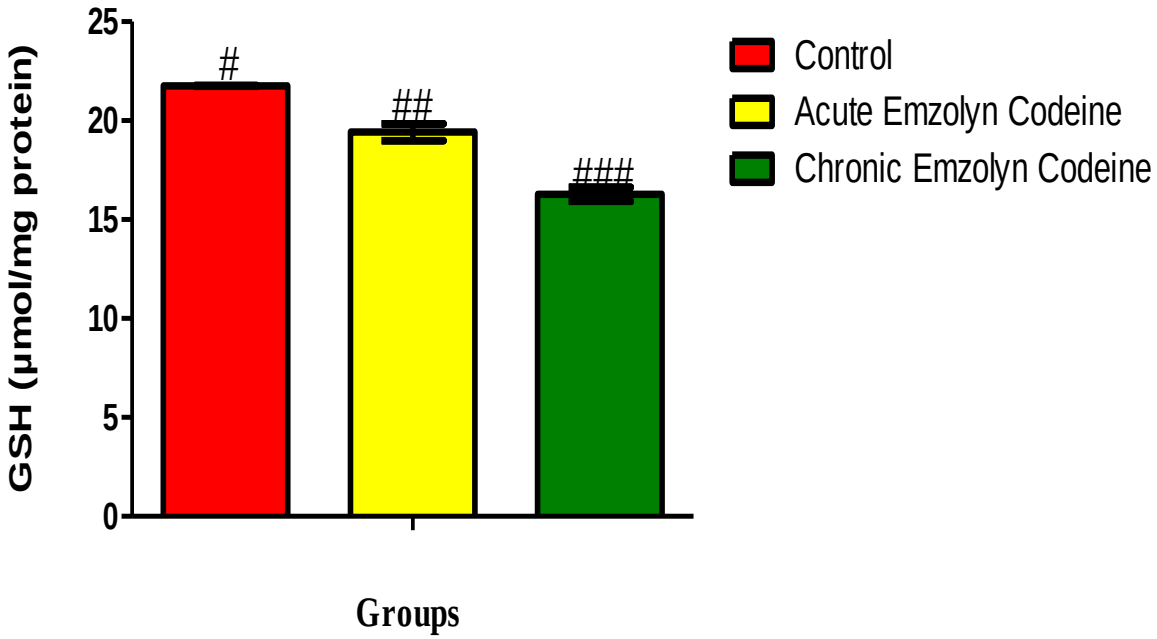

Fig. 5. Effects of Enzolym codeine on GSH Level in Normal Rats. Mean values with different sign are significantly different at P<0.05

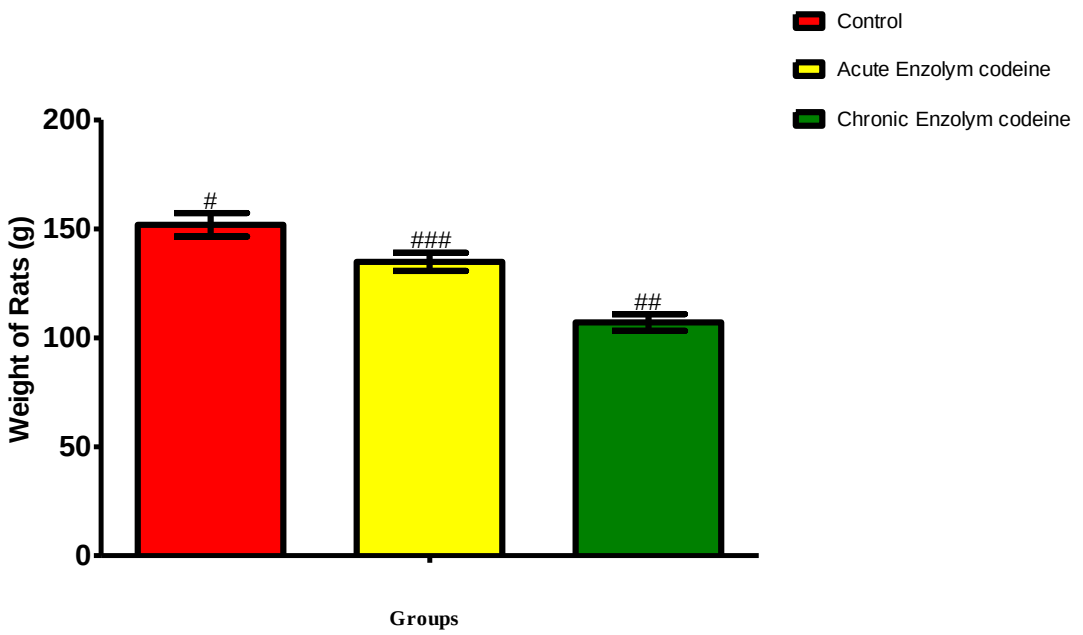

Fig. 6. Effects of Enzolym codeine on Body Weight of Rats. Data are shown as mean ± S.D (n=6). Mean values with different sign are significantly different at P<0.05

4. DISCUSSION

Emzolyn codeine is an analgesic mainly used to treat cough and to manage mild to moderate pain [9]. It is however, a drug of abuse because of its stimulatory effect on CNS among some adults [10]. Toxic effects of codeine use have been reported, although little is known about emzolyn codeine toxicity (Frost et al., 2012). In this study, histopathological effect of emzolyn codein cough syrup on lungs and its oxidative parameters were examined in animal models. Codeine was studied as a cough drug and not as analgesics because alarming misuse of codeine recently made Nigeria Government ban production and importation of cough syrup that has codeine as an ingredient [11]. Therefore, this study evaluated the toxicity of emzolyn codeine on systemic body organs because of people use of emzolyn codeine without doctor prescriptions.

The histological examination of the lungs of acute dose treated group showed edematous aveolar space with type II pnuemocyte and Hypertrophy of smooth muscle and blood while the chronic dose treated group showed edematous aveolar space with marked hypertrophy of the septa and marked inflammatory cells. This study is in agreement with the study of Lusk and Maloley, [12] whose study showed that usage of codeine is associated with pulmonary oedema. Apart from pulmonary oedema, there was bronchial smooth muscle hypertrophy which is characterized by increased size of smooth muscle cells and thickening of the smooth muscle layer around airways. It is a feature of airway wall remodeling in disease states resembling chronic asthma. Increased airway smooth muscle mass may contribute to bronchial narrowing and airway hyperresponsiveness [13].

In this study, there was a significant decrease in the activity of catalase, SOD, GPX and GSH in the exposed groups when compared to the controls, while mean levels of MDA were significantly increased. Oxidative stress results when there is increased production of free radicals or decreased activity of counter-actors, antioxidants or both in a combination (Suchitra et al., 2013). Antioxidant which can either be enzymatic (SOD, CAT, GPx) or non-enzymatic (GSH, TAS, Vitamin C, A etc) protects against effect(s) of free radicals in order to maintain homeostatic balance of reactive oxygen species [14]. SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen [14]. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in

protecting the cell from oxidative damage by reactive oxygen species (ROS) [15]. GPx is a selenium dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [16]. Significant reduction in SOD, CAT and GPx activity might be an indication of accumulation of H2O2 that required to mump up these reactive species. This study possibly depicts increased formation of free radicals that could lead to oxidative damage as a result of overwhelming antioxidant activities of all these enzymes. Glutathione had been reported to play a key role in maintaining proper function and preventing oxidative stress in human cells. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H2O2) dangerously reactive species within the cell [16]. Significant reduction in plasma levels of reduced glutathione (GSH) is thus a result of overwhelming antioxidant effects to reduce free radicals generated.

This finding suggests increase in oxidative stress, resulting in increased lipid peroxidation and this is corroborated by Pasupathi et al. [17] report. This study shows inverse correlation levels of significant MDA. The decreased in plasma SOD and CAT activity might be due to increased H2O2 production in exposed groups. The oxidative stress induced by codeine was reported by Lemarie and Grimm, [18]. They explained this by those complexes I, III, and IV of electron transfer chain in mitochondria were found to be inhibited by codeine at high doses. Inhibition of complex III resulted in the generation of ROS as a consequence of the intrinsic characteristics of the electron transfer process to this complex from reduced ubiquinone. The brain is particularly susceptible to oxidative damage due to its high levels of oxygen consumption, increased levels of polyunsaturated fatty acid and relatively low levels of enzymatic antioxidants [19]. Chronic administration of codeine to mice results in oxidative stress in brain tissues; and this effect was associated with a significant decrease in brain non-enzymatic antioxidant, intracellular reduced glutathione level and in enzymatic antioxidant, glutathione peroxidase activity [20].

The present study showed there was a significant (P≤0.05) decrease in body weight of emzolyn codeine treated group after the days of treatment. These could be due to a compromised nutritional status of the rats consequent on gastrointestinal tract derangement. This is in agreement with Thornhill et al. [21] who mentioned that codeine treated rats had a significant body weight loses (P<0.05) which had a greater weight loss of rats in (7.41%) when compared to the control animals, also the body weights decreased in rats exposed to (3.125) or (6.25) ppm of codeine and in mice exposed to (6.25) ppm of codeine were lower than those of the controls. While this study was in disagreement with Anurag et al. (2010) who reported that there was no significant change in the mean body weight of all the codeine treated groups as compared with control group for (30 days) [22-29].

5. CONCLUSION

In conclusion, this study observed that that emzolyn codeine cough syrup administration may cause decreased body weight, pulmonary toxicity and oxidative stress and as such, its use should be limited to prescription only.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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