Phytochemistry and Toxicity of three different Herbal Bitters (G. Winco, 1960 roots and Confam) on the Lungs of Wister Albino Rats

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Abstract: **This study evaluates the phytochemistry and toxicity of three different herbal bitters (G. Winco, 1960 roots and Confam) on the lungs of Wister albino rats. A total of forty (40) Wister albino rats were randomly divided into 4 groups labeled A, B, C and D and kept in a well ventilated room. Group A served as control and these rats were treated with distilled water. Rats in the groups B, C and D were treated with 3 different doses of the bitters (20, 30 and 40mL/Kgbw) respectively. The drugs were administered once daily for 10 and 21days consecutively. Animals were sacrificed 24 hours after the last treatment. Blood samples were collected into heparinized sample bottles for analysis. Phytochemistry done showed the presence of kaempferol, epicatehin, catechin and quinine amongst other components in varying amounts. Histopathological changes were found in all the bitters at the highest concentrations of 40ml/kg when used for both 10 days and 21 days. This shows that both short term and long term use of large quantities of herbal bitters affects the lungs negatively subsequently reducing gaseous exchange across the respiratory membrane**

Keywords: Phytochemistry, herbal bitters, Wister albino rats, histopathological changes.

I. INTRODUCTION

In the past few decades there has been an increase in the use of herbal/natural remedies in both developing and developed countries availability of modern medicines notwithstanding. In recent times there has been a paragon shift from orthodox medicine to traditional medicine in developing countries due to economic recession, low socioeconomic status of most of the population, absence of insurance and high cost of hospitalization [1]. These bitters are readily available, accessible and affordable to the common man. Also there is the erroneous belief and hope that one drug can cure and prevent all diseases.

In Nigeria, consumption of bitter drinks is gradually gaining grounds as a choice of natural remedy for the treatment of various diseases like indigestion, haemorrhoids, diabetes and prevention of colorectal cancer. They are also said to have anti-inflammatory, antibiotic and antifungal properties [1-3]. Some of the popular components of these bitters are orange peel, quinine, cassia etc. orange peel has been said to be anti-inflammatory, anti-microbial, antioxidant, aids digestion, and boosts theimmune system amongst other uses [4]. Quinine is known for its use as an antimalarial.

Cassia is an ayuverdic plant that is said to be antifungal, antiinlammatory, antimalarial, antioxidant, hepatoprotective, antibacterial and immunosuppressive [5]. Much has been done on the possible toxic effects of herbal bitters on organs such as the liver, kidney and cardiac function. The lungs are hardly ever studied in these studies. This caused the researchers to carry out this study on the phytochemistry and the toxicity of three different bitters on the lungs of Wister albino rats.

II. MATERIALS AND METHODS

Confam, G. Winco and1960 roots bitters used in this study was gotten from Mile 3 market, Diobu Port Harcourt. Specimen (animal) used for the experiment: forty (40) albino rats were purchased from animal house of the Department of Biochemistry , University of Port Harcourt, Choba Park. The animals were fed with rat pellets, water and libitum. Chemicals and reagents: all chemicals and reagents used in this study were obtained from Randox Laboratories UK. Preparation of Drug solution for administration: 20ml/kg, 30ml/kg and 40 ml/kg of the preparation was given to the rats each day after weighing depending on their respective groups.

Experimental procedure: a total forty (40) albino rats of weight range (124-194g/BW) were randomly divided into four groups labeled A, B, C, D and E where group A served as control and rats (n=2rats/dose) were treated with distilled water. Rats in groups B, C and D (n = 2 rats/dose) were orally treated with 3 different doses of Confam (20, 30 and 40ml/kgBW) , 1960 roots (20, 30 and 40ml/kgBW) and G. Winco (20, 30 and 40ml/kgBW) for 10 and 21days respectively. Animals were sacrificed twenty four (24) hours after last treatment.

2.1 Collection of Blood and Preparation of Serum

The rats were withdrawn from the cages in each of the group twenty four (24) hours after the last administration of the drugs for 10 and 21 days and placed in a desiccator containing cotton wool soaked in chloroform to anaesthetize the rats. The blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade and put in anticoagulant sample bottles smeared with lithium-heparin and fluoride oxalate. The blood samples were spun at

5000rpm using MSE Centrifuge to obtain plasma. The animal was dissected and only the lung was collected for pathological studies.

2.2 Histological Procedures and Analysis

The lung was cut on slabs about 0.5cm thick and fixed in 10% normal saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20mins each in an oven at 57%. Several sections of the 5µm thick were obtained from a solid block of tissue and were stained with hematoxylin and eosin staining after which they were passed through a mixture of equal concentration of xylene and alcohols, following clearance of xylene, the tissues were oven dried. Photomicrographs were taken with a JVC colour video digital camera (JVC China) mounted on an Olympus light microscope (Olympus UK Ltd Essex, UK) to demonstrate cytoarchitecture of the lung.

III. RESULTS AND DISCUSSION

Phytochemistry analysis showed same components in all three bitters at varying amounts. Some of these are kaempferol, epicatehin, catechin, quinine, lunamarin, flavones, phytate amongst others.

3.1 Results for day 0 administration

Results of the phytochemical analysis of the different bitters are presented in Table 1. Results obtained indicated that quercetin and rutin were absent in Confam, 1960 roots and G. Winco bitters while anthocyanin was not found in G. Winco bitter.

Sapogenin was absent in 1960 roots while flavones was not found in 1960 roots and G. Winco respectively. There was no oxalate in 1960 roots but present in G. Winco and Confam at varying concentrations respectively. Figure 1 shows that the photomicrograph of lung tissue treated with distilled water showed normal alveolar sacs and interalveolar septa.

Table 1: Phytochemistry of the three bitters (comfam, 1960 roots and G. Winco)

Parameter	Confam bitter	1960 roots	G. Winco bitter
Quercetin, µg/g	-	-	-0
Kaempferol, µg/g	3.0262	3.2011	5.5555
Epicatechin, µg/g	25.7789	22.8281	17.7500
Catechin, µg/g	3.5072	6.2998	8.2304
Anthocyanin, µg/g	17.5513	4.4307	-
Sapogenin, µg/g	8.9334	-	13.1295
Ribalindine, µg/g	3.3548	16.3195	8.0570
Rutin, µg/g	-	-	-
Tannin, µg/g	18.2080	15.4276	15.6713
Anthocyanidines, µg/g	0.3112	0.2653	0.3676
Spartein, µg/g	6.6264	23.7048	9.0634
Lunamarin, µg/g	10.8255	4.6041	7.3398
Quinine, µg/g	22.0149	11.0964	6.4907
Oxalate, µg/g	7.7015	-	3.5596
Naringin, µg/g	6.3765	2.9438	2.3244
Flavones, µg/g	2.1605	-	-
Phytate, µg/g	5.2622	3.2181	9.4486

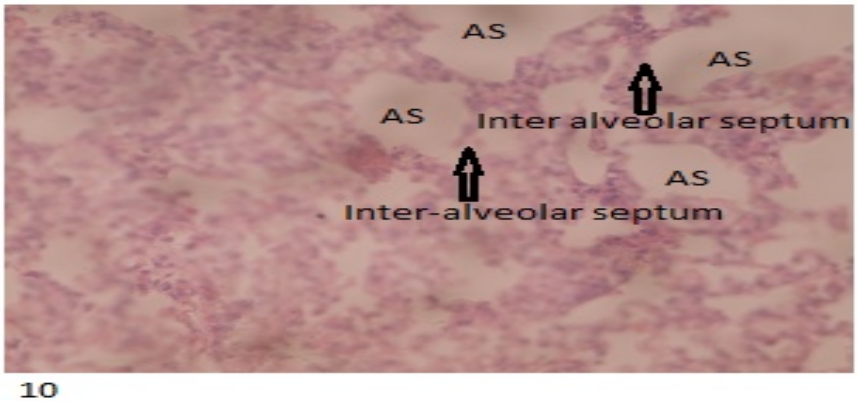
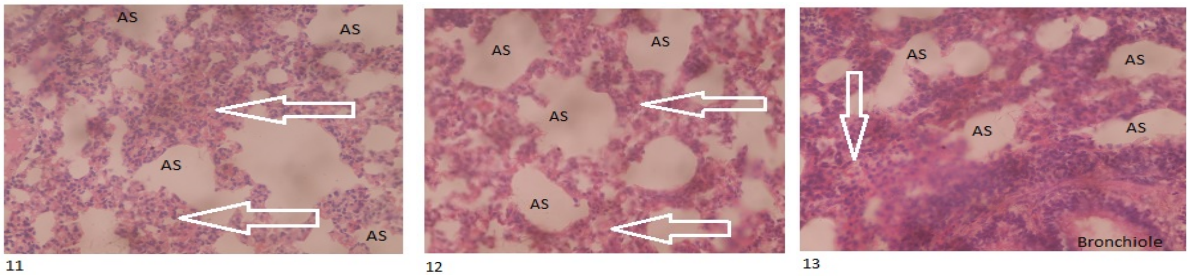


Figure 1: Photomicrograph of lung tissue treated with distilled water (control).

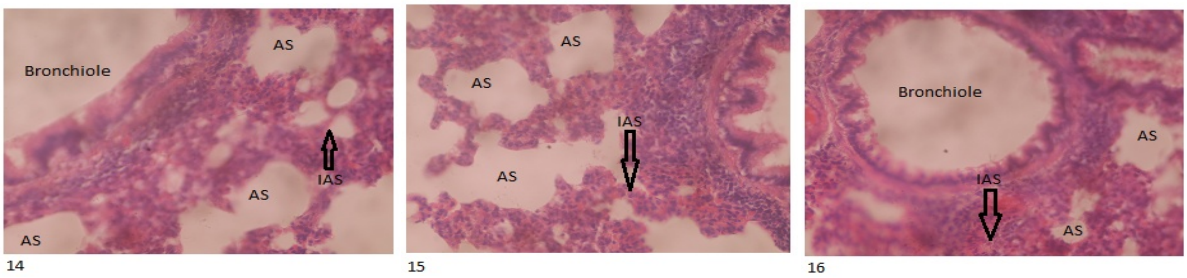
3.2 Results for 10 days of administration

Figure 2: photomicrograph of lung tissue treated with confam 20ml/kg showed normal alveolar sacs and thickened interalveolar septa and blood vessels. Figure 3: photomicrograph of lung tissue treated with confam 30ml/kg showed normal alveolar sacs and thickened interalveolar septa. Figure 4: photomicrograph of lung tissue treated with confam 40ml/kg showed normal alveolar sacs, a bronchiole and thickened interalveolar septa.



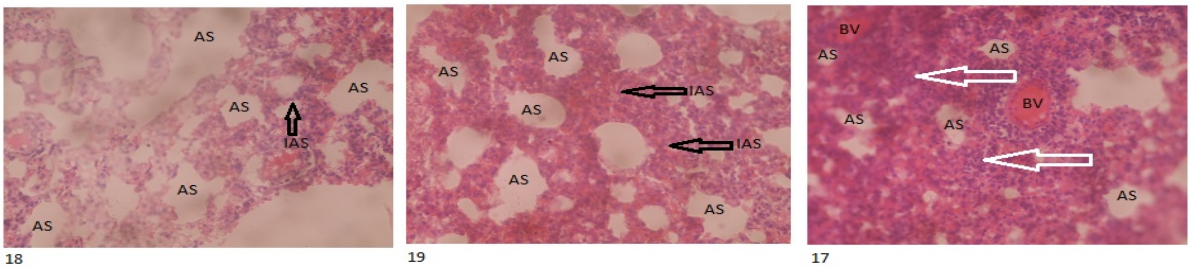
Figures 2, 3 and 4 (L-R)

Figure 5: photomicrograph of lung tissue treated with 1960 roots 20ml/kg showed normal alveolar sacs, interalveolar septa and bronchiole. Figure 6: photomicrograph of lung tissue treated with 1960 roots 30ml/kg showed normal alveolar sacs, interalveolar septa and bronchiole. Figure 7: photomicrograph of lung tissue treated with 1960 roots 40ml/kg showed normal alveolar sacs, blood vessels, a bronchiole and thickened interalveolar septa.



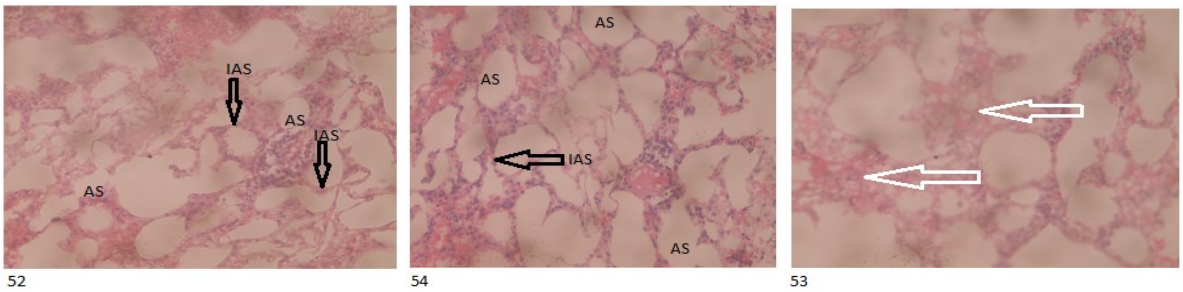
Figures 5, 6 and 7 (L-R)

Figure 8: photomicrograph of lung tissue treated with G. Winco 20ml/kg showed normal alveolar sacs, interalveolar septa and blood vessels. Figure 9: photomicrograph of lung tissue treated with G. Winco 30ml/kg showed normal alveolar sacs and thickened interalveolar septa. Figure 10: photomicrograph of lung tissue treated with G.winco 40ml/kg showed alveolar sacs that were decreased in size, congested blood vessels and thickened interalveolar septa.



Figures 8, 9 and 10 (L-R)

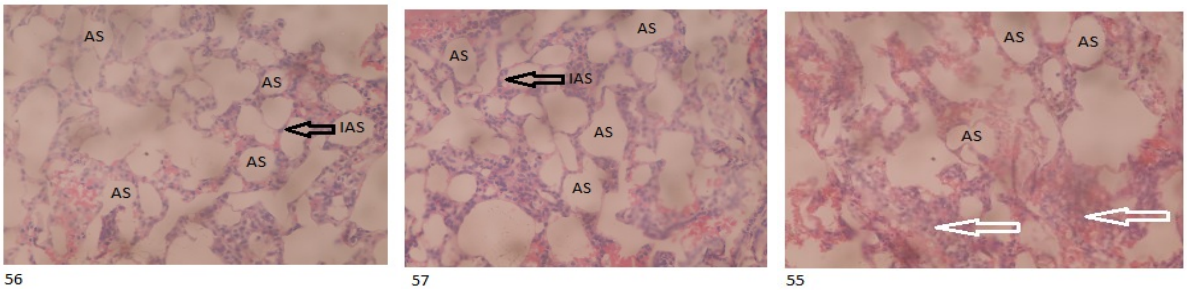
Figure 11: photomicrograph of lung tissue treated with confam 20ml/kg showed normal alveolar sacs and interalveolar septa. Figure 12: photomicrograph of lung tissue treated with confam 30ml/kg showed normal alveolar sacs, blood vessels and interalveolar septa. Figure 13: photomicrograph of lung tissue treated with confam 40ml/kg showed normal alveolar sacs and some thickened interalveolar septa (shown by arrows).



Figures 11, 12 and 13 (L-R)

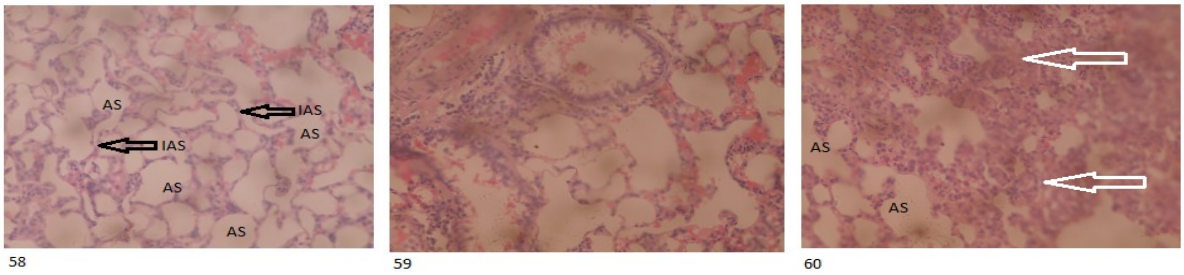
3.3 Results of 21 days of administration

Figure 14: photomicrograph of lung tissue treated with 1960 roots 20ml/kg showed normal alveolar sacs and interalveolar septa. Figure 15: photomicrograph of lung tissue treated with 1960 roots 30ml/kg showed normal alveolar sacs and interalveolar septa. Figure 16: photomicrograph of lung tissue treated with 1960 roots 40ml/kg showed normal blood vessels, some collapsed alveolar sacs and some thickened interalveolar septa (shown by arrows).



Figures 14, 15 and 16 (L-R)

Figure 17: photomicrograph of lung tissue treated with G. Winco 20ml/kg showed normal alveolar sacs and interalveolar septa. Figure 18: photomicrograph of lung tissue treated with G. Winco 30ml/kg showed normal alveolar sacs, a bronchiole and interalveolar septa. Figure 19: photomicrograph of lung tissue treated with G. Winco 40ml/kg showed normal alveolar sacs and thickened interalveolar septa.



Figures 17, 18 and 19 (L-R)

The results in Figures 1 to 19 are similar to those obtained in a the research done by Aniagu *et al.* [6] which showed related increase in lungs weight after exposure to a bitter. These changes are more noticed when the bitters are used in large doses [7-12]. Thickened interalveolar septum reduces gaseous exchange at the respiratory membrane [13-16]. This is dangerous as it causes hypoxemia, ischemia and finally cell death. This can be manifested in various ways

IV. CONCLUSION

Results at 10 days of administration showed thickened interalvolar septa in all concentrations of confam bitters given. G. Winco bitters showed thickened interalveolar septa from 30ml/kg of administration. At the highest concentration there was also congestion of blood vessels and decrease in the size of alveolar sacs. 1960 roots had thickened interalveolar septa at the 40ml/kg dose.

At 21 days of administration, all the bitters had thickened interalveolar septum at only the highest dose which

is 40ml/kg. 1960 roots also had some collapsed alveolar sacs at this dose. This result shows that irrespective of duration and the type of bitters used, they all cause pathological changes in the lungs. Adequate dilution of these bitters should be made before their consumption.

REFERENCES

[1] Hussain, I, Ullah, R, Khurram, M, Ullah, N, Baseer, A, Khan, FA, Khattak, MR, Zahoor, M, Khan, J and Khan, N. (2011). Phytochemical analysis of selected medicinal plants. *African Journal of Biotechnology*, 10(38): 7487-7492.

[2] Jimmy EO. Udofia AJ. (2010). Yoyo bitters, a potent alternative herbal drug in the treatment of diabetes. International Journal of Innovative Medicine and Health Science, l2:1-5.

[3] Osamor PE, Owumi BE. Complementary and alternative medicine in the management of hypertension in an urban Nigerian community. *BMC Complement Altern Med.* 10(1): 36-44.

[4] Ould Yerou K., Ibri K., Bouhadi D., Hariri A., Meddah B., Tir Touil A. (2017). The use of orange (*citrus sinensis*) peel as antimicrobial and antioxidant agent. *Journal of Fundamental and Applied Sciences*, 9(3), 1351-1357.

[5] Vijay AS, Ranjitha J, Devi RV, Bhagiyalakshmi M. (2013). Pharmalogical profile of cassia occidentalis l- a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5 (3): 29-33

[6] Aniagu S., Nwinyi CF, Akumka D.D., Ajoku AG, Dzarma S, Izebe KS, Ditse M, Nwaneri EC, Wambebe C, Gamaniel K. (2005). Toxicity studies in rats fed nature cure bitters. *African Journal of Biotechnology*, 4(1):72-78.

[7] Falodun A. (2010). Herbal medicine in Africa - distribution, standardization and prospects. *Research Journal of Phytochemistry,* 4: 156-161.

[8] Ogechi, N.E. andIbioku, E. (2018). Evaluation of the effect of action bitters (herbal mixtures) on some biochemical indices of albino rats. *Asian Journal of Research in Biochemistry*, 3(4) 1-8.

[9] Oforibika, A. G. and Uwakwe, A. A. (2020). Comparative effects of three antiretroviral drugs on liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). *European Journal of Pharmaceutical and Medical Research*, 7(1): 53-57.

[10] Budak HN, Kumbul Doguc D, Savas CM, Seydim AC, K¨ok Tas T, Ciris IM, G¨uzel-Seydim ZB. (2011). Effects of apple cider vinegars produced with different techniques on blood lipids in high-cholesterol-fed rats. *J Agric Food Chem.* 59:6638–44.

[11] Lim S, Yoon JW, Choi SH, Choa BJ, Kim JT, Chang HS, Park HS, Park KS, Lee HK, Kim YB, Jang HJ. (2009). Effect of ginsam, a vinegar extract from Panax ginseng, on body weight and glucose homeostasis in an obese insulin-resistant rat model. *Metabolism,* 58:8–15.

[12] Chang J, Fang TJ. (2007). Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars *typhimurium* in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. *Food Microbiol.* 24:745–51.

[13] Iriti M, Faoro F. (2010). Bioactive chemicals and health benefits of grapevine products. In:Watson RR, Preedy VR, editors. Bioactive foods in promoting health: fruits and vegetables. New York: Elsevier Inc. p 581–20.

[14] Oforibika, A.G. And Ezekiel, T. (2017). Hepatotoxicity of Lamivudine on the liver. *Biomedicine and Nursing*, 3(2):49-52.

[15] Ogechi, N.E. andIbioku, E. (2018). Evaluation of the effect of action bitters (herbal mixtures) on some biochemical indices of albino rats. *Asian Journal of Research in Biochemistry*, 3(4) 1-8.

[16] Oforibika, A. G. and Uwakwe, A. A. (2020). Comparative effects of three antiretroviral drugs on liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). *European Journal of Pharmaceutical and Medical Research*, 7(1): 53-57.