Original Article

Effect Of Folic Acid And Cobalamin Supplementation On Collagen And Elastin Content In Rats' Lung Tissue: An Experimental Study

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Abstract

Objective: The objective of this study was to investigate how the supplemental use of Folic Acid and Cobalamin impacts the levels of collagen and elastin in the lung tissue of female Sprague-Dawley rats.

Study Design: Laboratory-based Experimental Study

Place and Duration of Study: This study was carried out at the anatomy department of the CMH Multan Institute of Medical Sciences Pakistan from September 2020 to September 2021.

Methodology: In this study, one hundred and twenty female Sprague-Dawley rats were used and divided into four groups. Each group comprised thirty adult female rats. Group A received a daily oral gavage of 1ml/100g of pure distilled water for four weeks. Group B1 received a daily oral gavage of 0.2mg/kg of folic acid in 1ml/100g of pure distilled water for four weeks. Group B2 underwent a daily oral gavage of 45µg/kg of cobalamin in 1ml/100g of pure distilled water over four weeks. In the case of Group B3, both folic acid (0.2mg/kg) and cobalamin (45µg/kg) were administered as supplementary doses in 1ml/100g of distilled water by oral gavage daily for four weeks. After 28 days of drug administration, the rats from all four groups were sacrificed. Sections were processed, and slides were stained with Masson's Trichrome and aldehyde fuchsin for a light microscopic study. Data was entered and analysed via SPSS version 26.

Results: The study revealed statistically significant differences in collagen and elastin content between the control and experimental groups. Specifically, the experimental groups showed an increased percentage of collagen and a decreased percentage of elastin compared to the control group.

Conclusion: This experimental study shows that long-term administration of supplementary Folic Acid and Cobalamin doses brings about changes in the collagen and elastin content of lung tissue.

Key Words: Folic acid, Cobalamin, Collagen, Elastin, Lung tissue

INTRODUCTION

The water-soluble B vitamins are essential coenzymes that are required for optimal cellular function. The group of vitamins known as Vitamin B consists of Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxal (B6), Biotin (B7), Folate (B9), and Cobalamin (B12). [1] Folic acid and Cobalamin are essential components of a balanced diet. In cells undergoing chromosomal replication and division, a deficiency in either of these vitamins can impede DNA synthesis. [2]

The recommended dietary allowance of folate and Cobalamin for an adult person is 400 and 2.4 micrograms per day, respectively. [3] Due to their importance, food is fortified with these vitamins. However, fortifying has led to a widespread increase in folic acid and Cobalamin intake that exceeds the body's physiological requirements. Serum or plasma folate levels exceeding 45nmol/L (19.8 ng/mL) in blood samples obtained after fasting are frequently categorised as supraphysiological. Findings from the National Health and Nutrition Examination Survey conducted between 1999 and 2000 indicated that around a quarter of the population exhibited serum folate concentrations considered supraphysiological. [4]

Folic acid (Pteroyl glutamic acid) is made up of a pteridine ring, para-aminobenzoic acid, and glutamate, all of which are connected by a methylene bridge. Folic acid itself does not possess biological activity. However, it converts into its active forms in the liver, namely dihydrofolic acid and tetrahydrofolic acid. [5] Tetrahydrofolate is a part of the one-carbon metabolism process, which is what makes thymidylate, purines, and pyrimidines, which are needed for DNA replication. The amino acids serine, histidine, and glycine primarily contribute this one-carbon unit to tetrahydrofolate. The attachment of these carbon units to tetrahydrofolate enables it to be either oxidised or reduced. By donating a carbon atom from its side chain to folate, serine aids in the transformation of serine into glycine and tetrahydrofolate (THF) into methylene tetrahydrofolate (mTHF). Methylene tetrahydrofolate (mTHF) is a carbon donor in the methionine cycle. [6] Cobalamin is the largest of the B-complex vitamins. It comprises a porphyrin ring composed of four pyrroles, similar to haem, with the distinction that cobalt is positioned at the center of the ring instead of iron. [2] Cobalamin is necessary for the normal metabolism of folate because it acts as a cofactor in the methionine cycle. [7]

Folic acid and Cobalamin have extensive applications in the treatment of megaloblastic anaemia and as preventive measures against neural tube defects (NTDs). [8] Food is fortified with folic acid and cobalamin in many countries, including Pakistan, to prevent nutritional deficiencies. [9, 10] A recent study revealed that excessive supplementation with Folic acid and Cobalamin is linked to alterations in the DNA methylation of multiple genes potentially associated with carcinogenesis. [11] A retrospective cohort study identified a correlation between high-dose supplementation of vitamin B6 and Cobalamin and an elevated risk of lung cancer. [12]

Lung cancer is the second most common cancer in terms of incidence and ranks first in terms of cancer-related mortality. Lung cancer is common in both males and females, but historically, it has been more prevalent in males. However, in recent years, the gap in lung cancer rates between genders has been narrowing, and the incidence of lung cancer in females has been increasing. [13] This study had two main goals. Firstly, this study was conducted to investigate the association between these vitamins and lung cancer since no experimental studies have been undertaken to confirm their relationship, aiming to bridge the knowledge gap. Secondly, the study aims to quantify the collagen and elastin components of lung tissue in histology slides with image J.

Material And Method

The research investigation took place within the research laboratory of the Department of Anatomy at the CMH Multan Institute of Medical Sciences, Pakistan. One hundred and twenty healthy female Sprague-Dawley rats were obtained from The University of Veterinary and Animal Sciences (UVAS) Lahore, a renowned research institute known for its veterinary laboratories. Sprague-Dawley rats are widely recognized as highly suitable experimental models and are extensively used in research globally. [14]

Pregnant rats and rats exhibiting any physical deformities were excluded from the study. The selection of rats was based on a random sampling method. The study included four groups: one control (A) and three experimental (B1,2,3). Each group comprised thirty adult female rats. Group A received a daily oral gavage of 1ml/100g of pure distilled water for four weeks. Group B1 received a daily oral gavage of 0.2mg/kg of folic acid in 1ml/100g of pure distilled water for four weeks. Group B2 underwent a daily oral gavage of 45µg/kg of cobalamin in 1ml/100g of pure distilled water over four weeks. In the case of Group B3, both folic acid (0.2mg/kg) and cobalamin (45µg/kg) were administered as supplementary doses in 1ml/100g of distilled water by oral gavage daily for four weeks. With the assistance of a reference article and a pilot procedure, we selected the optimum dose. [15, 16] Control and experimental animals were kept under the same conditions. Day 0 was considered the first day of the experiment. After 28 days of drug administration, the rats from all four groups were sacrificed. The weight of all the animals was taken before sacrifice. The rats were euthanized by chloroform inhalation, and when the rats showed no respiratory or body movements, they were placed on a clean sheet of paper on dissecting board for dissection. [17] Skin and muscle covering the ventral thoracic and cervical regions were removed using scissors and forceps. Proceeded by making two lateral cuts on each side of the ribcage and an additional one near the clavicle to open up enough space for a comprehensive examination of all lung lobes. This will expose the heart and lungs for further observation. Hold the trachea close to the jaw using forceps, then make a complete cut through the trachea using scissors positioned above the forceps. Delicately elevate the trachea with the forceps, carefully severing tissue connections on the underside using scissors, until the complete assembly of thoracic tissues (including the trachea, lungs, and heart; often referred to as the "pluck") has been successfully extracted from the body. Lay the lungs flat on the work surface. Place the lungs into fixative using an approximate 20:1 fixative-to-tissue ratio. A transverse section of the left lobe was taken for histopathology. [18, 19] Sections were processed, and slides were stained with Masson's Trichrome and aldehyde fuchsin for a light microscopic study. Slide photographs were taken with a 40X objective lens using the Adobe Photoshop Lightroom version 20.0.4 application on a mobile camera. The percentage of collagen fibres per unit area and the percentage of elastic fibres per unit area in the lung tissue were measured using Image-J software, a public-domain, Java-based image processing software developed at the National Institute of Health (USA) in 1997. The Color Thresholding Method was applied to quantify Collagen and Elastin. Hong et al. demonstrated the quantification of collagen or elastin visible in histological photographs using ImageJ,based on their unique colour.[20] For this study, ImageJ was opened, and its scale was calibrated by opening the image of the stage mmicrometre taken with the same camera and magnification settings as the other photographs. Next, Analyze > Set Measurements was accessed, and 'Area' and 'Limit to Threshold' were selected while deselecting other measurements. Afterwards, the image was opened for measurement, ensuring it was captured at the same magnification and camera system as the stage mmicrometrephotograph used for scale calibration. To measure the area of the entire picture, Analyze > Measure or the keyboard shortcut 'Ctrl + M' was utilized, displaying the results in a separate window. The Hue, Saturation, and Brightness were adjusted using the default method by going to Image > Adjust > Color Threshold to accurately capture the color of interest, such as blue in the Masson Trichrome staining. Subsequently, Analyze > Measure or 'Ctrl + M' was used again to calculate the area of the selected region in calibrated units, and the results were recorded. Finally, with the measurements taken, the percentage of collagen in the images was calculated by applying the formula. The same method was applied to quantify the elastin component. The data was expressed as mean ± S.D (Standard Deviation). A one-way ANOVA followed by the post-hoc Tukey test was applied to quantitative variables to detect any significant differences between the experimental and control groups.

RESULTS

The study revealed significant differences in collagen and elastin content between the Control and experimental groups. Specifically, the experimental groups showed an increased percentage of collagen and a decreased percentage of elastin compared to the control group, as shown in Figure I.

The percentage area of collagen per unit area in rats' lung tissue (%) was calculated for both the control group and the experimental groups. The mean percentage area of collagen in lung tissue of Group A (Control) was 12.57 ± 9.17 %, for B1 (Folic Acid) it was 18.63 ± 8.35 %, for B2 (Cobalamin Group) 21.47 ± 10.00 %, and for B3 (F.A. + Cobalamin Group) 23.77 ± 10.30 %. The overall ANOVA showed a significant difference between the Control and Experimental groups ($p = 0.000$). (Figure II)

Pair-wise comparison by post hoc Tukey test showed that the group A (control)'s percentage of collagen per unit area in the lung tissue was significantly lower than the B2 (cobalamin) ($p = 0.002$) and B3 (f.a. + cobalamin) groups ($p = 0.000$).

The percentage area of elastin per unit area in rats' lung tissue (%) was calculated for both the control group and the experimental groups. The mean percentage area of elastin in lung tissue of Group A (Control) was 24.80 ± 7.78%, for B1 (Folic Acid) it was 6.17 ± 3.84%, for B2 (Cobalamin Group) 8.10 ± 3.45%, and for B3 (F.A. + Cobalamin Group) 8.00 ± 4.18%. The overall ANOVA showed a significant difference between the groups ($p = 0.000$). (Figure III)

Pair-wise comparison by post hoc Tukey test showed that the group A (control)'s percentage of elastin per unit area in the lung tissue was significantly higher than that of B1 (folic acid) ($p = 0.000$), B2 (cobalamin) ($p = 0.000$), and B3 (F.A. + cobalamin) groups ($p = 0.000$).

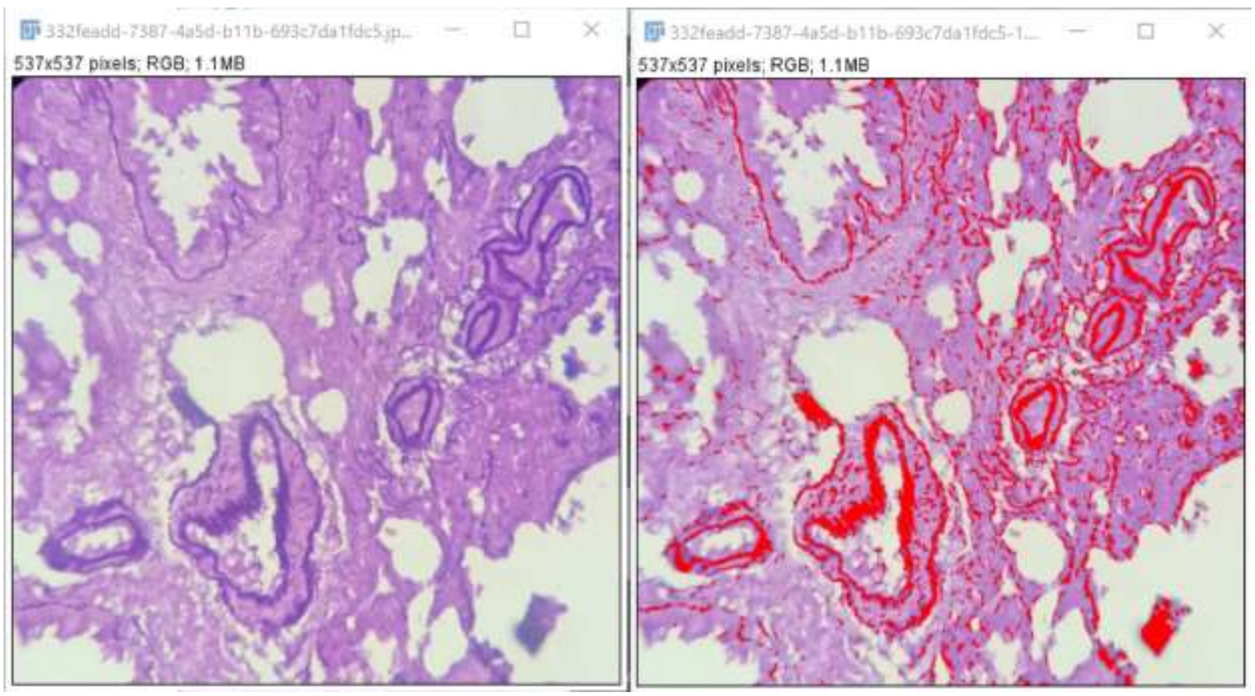

Figure I: Quantification of the elastin component using ImageJ software.

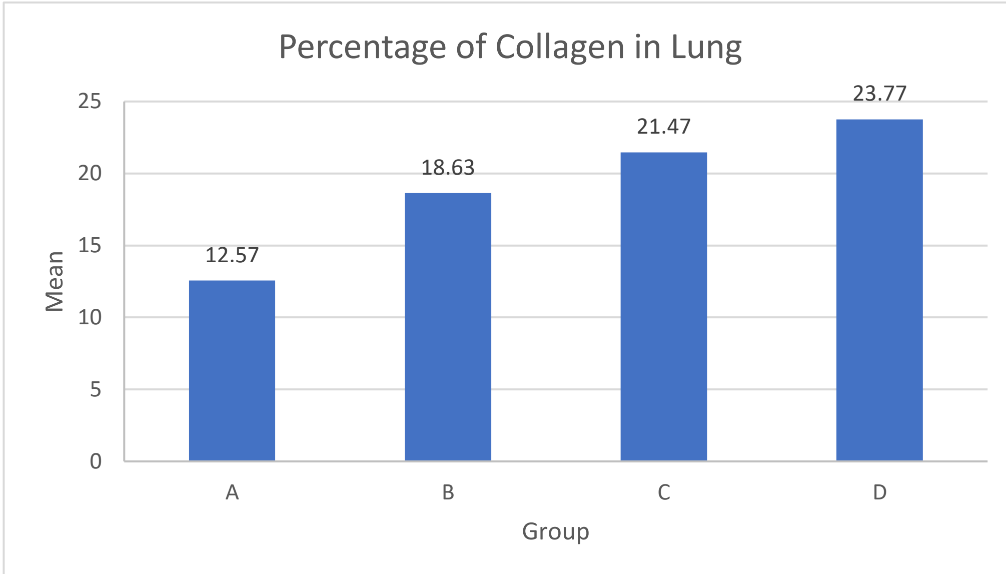

Figure I I: Mean percentage of collagen per unit area in lung tissue

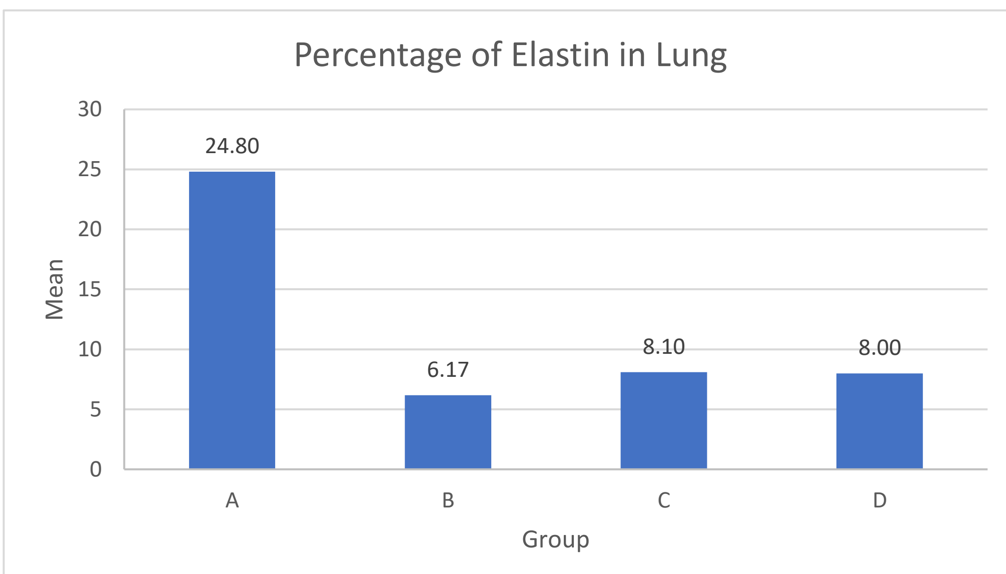

Figure III: Mean percentage of elastin per unit area in lung tissue

DISCUSSION:

The present study investigated the effect of folic acid and cobalamin supplementation on collagen and elastin content in rats' lung tissue using an experimental approach and image analysis. The water-soluble B vitamins, folic acid, and cobalamin are essential coenzymes required for optimal cellular function. [1, 2] They play crucial roles in DNA synthesis and chromosomal replication. Despite their importance, excessive supplementation has become a concern due to widespread fortification of foods, leading to supraphysiologic serum folate concentrations in a significant portion of the population. [4]

The results revealed significant differences in collagen and elastin content among the groups. The experimental groups (B1, B2, and B3) showed altered percentages of collagen and elastin compared to the control group (Group A). Group B1 (folic acid) exhibited a higher percentage of collagen, while Groups B2 (cobalamin) and B3 (folic acid + cobalamin) showed even higher collagen content compared to the control. On the other hand, Group A had a significantly higher percentage of elastin compared to the experimental groups, indicating a potential role of folic acid and cobalamin in modulating elastin content in lung tissue.

The findings of an increased collagen component in the experimental groups are consistent with previous studies showing that folic acid and cobalamin are involved in one-carbon metabolism, which is essential for DNA synthesis and cellular division. [21] The alteration in the collagen component can impact the lung's ability to heal and adapt to various conditions, potentially affecting overall lung health and function. However, further investigations are required to understand the mechanisms underlying this effect.

Conversely, the decrease in elastin content observed in the experimental groups might have important implications for lung function and elasticity. Elastin is a crucial component of lung tissue, allowing it to expand and recoil during the breathing process. [22] The decreased elastin content impacts lung compliance and respiratory function, warranting further investigation into its physiological significance.

The association between folic acid and cobalamin supplementation and lung cancer has been a subject of interest in recent research. Some studies have reported potential links between high-dose supplementation of vitamins B6 and B12 and lung cancer risk. [23, 24] In the context of this study, it is essential to consider the potential impact of altered collagen and elastin content on lung tissue integrity and function, as these components play significant roles in maintaining the structural integrity of the lungs. [25]

However, it should be noted that this study has some limitations. Firstly, the experiment was conducted on female rats, and gender-specific effects cannot be ruled out. Secondly, the study period was limited to four weeks, and the long-term effects of folic acid and cobalamin supplementation were not explored.

These findings contribute to the ongoing research on the potential health consequences of excessive folic acid and cobalamin intake and their relevance to lung cancer risk. Future studies may explore gender-specific effects and long-term outcomes to provide a comprehensive understanding of the impact of these essential B vitamins on lung health.

CONCLUSION

This experimental study shows that long-term administration of supplementary Folic Acid and Cobalamin doses brings about changes in the collagen and elastin content of lung tissue.

Conflicts OF Interest

There are no conflicts of interest.

SOURCE OF FUNDING

None

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