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# Pulmonary Effects of Dexfenfluramine

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**Abstract:** Dexfenfluramine is a variation of fenfluramine which is used for weight reduction. The aim of the study is to determine the hemodynamic and structural effects of this drug on pulmonary circulation.

An experimintal study was carried out with New Zealand Rabbits. A control group (n=30) and an experimintal group (n=20) was constituted. A four-week normal diet for control group and dexfenfluramine plus normala diet for experimental group were applied. Body weights and pulmonary artery pressures of groups were recorded and lungs were collected for pathologic examination.

In experimintal group; Dexfenfluramine raised systolic pulmonary artery pressures significantly ($p<0.01$), whereas, there were no meaningful changes on diastolic and mean pulmonary artery pressures ($p>0.05$). Weight reduction was obvious ($f= 8.522$, $p<0.01$), in experimental group. There were marked histopathologic changes in accordance with pulmonary hypertension. In addition, squamouse metaplasia (n=1) and tumorlet (n=3) formations were seen.

The use of dexfenfluramine was associated with the development of primary pulmonary hypertension. Duration and dosage relations of the therapy and topical effects of the drug should be investigated with further studies. The reasons of tumorlet formation and squamouse metaplasia must be explained with new researches.

**Key Words:** Dexfenfluramine, fenfluramine, obesity, appetite depressants, pulmonary hypertension.

## Introduction

Dexfenfluramine (Isomeride®) is a new drug in the market that is still underinvestigation in many countries. Recently, it is largely used in our country for weight reduction.

Dexfenfluramine is an fenfluramine derivative that used as an appetite supressant. It is an antiobesity drug. Dexfenfluramine is a dextro- rotalary (+) stereoisomer of fenfluramine that is a pure serotonin (5-hydroxytryptamine; 5Ht) agonist devoid of dopaminergic or sympathominetic activity (1).

Dexfenfluramine is generally regarded by clinicians as a safe medication. We tried to find out pulmonary effect of it. Eary phases of therapy, mild and transitory side effects of the drug have been demonstrated (2,3). Recent studies emphasize the risk of primary pulmonary hypertension of appetite-supressant drugs such as; derivatives of fenfluramine (fenfluramine and dexfenfluramine), amphetamine-like anorexic agents (diethylpropion [amfepramone], clobenzorex, fenproporex, mazindol and phenmetrazine) (4). In addition, rare cases of pulmonary hypertension and probability of neurotoxicity have been reported (5-7).

We sought the pulmonary paranchymal and vascular effects of dexfenfluramine.

## Materials and Methods

### Animals

An experimental study was carried out on New Zealand Rabbits. Pulmonary artery pressures were obtained directly on living subjects with a cardiac ECG-pressure monitor (Fig. 1). It was rather an invasive procedure. Lack of post-operative cara units, animals were sacrificed after measurements. A control group (n=30) and an experimental group (n=20) were created. Body weights of groups were equal ($p<0.01$). Body weights were between 2410 and 3150 g. (Mean= 2753g.) (Table 1). All animals received care in

compliance with the "Principles Of Laboratory Animal Care" formulated by the National Society for Medical Research.

### Study Design

The animals were anaesthetized with Rompun" (Xylazin Hydrochlorid) 15 mg/kg IM. and Ketalar, (Ketamine Hydrochlorid) 60 mg/kg IM. At the beginning, tracheostomy was performed and a 12G cannula was inserted into the trachea. Then sternotomy was performed and lungs were ventilated with a small ambu-like device. After pericardiotomy, pulmonary artery pressures were measured directy with a needle from pulmonary infindibulum (Fig. 1).

### Methods

The drug should be given orally because there are no other pharmacological forms. Control group was fed with normal diet, experimental group was fed with dexfenfluramine added normal diet for four weeks. Gastric content was seen then dexfenfluramine (7.5mg/kg/day) was given once in a day via nasogastric way. Animals which were traumatized during this application were excluded from the study. At the beginning, during the 2nd and the 4th weeks of the treatment, body weights were measured (Table 1). To put aside the stress from nasogastric feeding, two days later from the last measurement, pulmonary artery pressures were recorded. Lungs of 10 animal from control group and all from the experimental group were removed as previously mentioned.

Lung tissues were sampled one midline section from both the right and left lungs. The tissues were immersed in formalin (10%) fixative. An average biopsies were yielded (4 slices). Each slice was dehydrated and embedded in parafin. 5 micron-thick sections were stained with hematoxylin-eosin, and orcein stain for elastic fibers.

The histologic sections were examined systematically changes in bood vessels and paranchyma were recorded by two pathologists (RE-EB) who were unaware of the clinical findings. Preaciner and intraaciner arteries were scored for severity of medial hypertrophy, intimal thickening and presence of focal paranchymal lesion. Morphometric analysis was not performed. Positive results were determined when the changes seen more than 25%.

Based on the predominant histopathologic lesions, the groups (control and experimental) were classified into three groups (Table 2). Classification was done by the two pathologists (RE-EB) without the knowledge of hemodynamic parameters.

### Data and Statistics

Pulmonary artery pressures were obtained as

Table 1. Body weights and pulmonary artery pressures of groups.

| | Control Goup (n=30) | Experimental Group (n=13) | P values |
|---|---|---|---|
| Body Weight (gr). | | | |
| At the beginning (1st week) | 2753.33±32.3 | 2721.54±60.1 | p>0.05 |
| Middle (2nd week) | 2823.33±33.1 | 2625.38±71.2 | p>0.05 |
| End (4th week) | 2906.67±34.4 | 2443.08±74. | p<0.01 |
| Pulmonary Artery Pressures (mmHg) | | | |
| Systolic | 21.5±0.7 | 24.77±0.7 | p<0.01 |
| Diastolic | 21.5±0.7 | 24.77±0.7 | p>0.05 |
| Mean | 16.6±0.6 | 16.23±0.9 | p>0.05 |

mmHg with a transducer (DELTRAN II, Utah Medical Products, Inc. USA) and a recorder (cardiac ECG-Pressure monitor, SCHİLLER CH-6340 BAAR, Schiller AG., Switzerland).

Body weights were determined as gram using a bascul (BASTER TBT-5, BASTER Ltd., TURKIYE).

Lungs were examined with a light microscope (NIK-ON, HF X-II A, optiphot-2, Japar)

Results are presented as the mean and standard error of the mean. Overall significance of differences between groups was determined by a ANOVA test using Epi Info 5.0 pc program.

## Results

7 animals in experimental group were excluded from the study due to accidental respiratory insertion (n=3), esophageal perforation (n=2) and hypoxic arrest (n=2) during nasogastric insertion. Body weights were significantly reduced at the 4th week regarding 13 animals' results. Mean body weights were 2721±60 g. and 2443± 74 g. ($F=8.522$, $p<0.01$) at the first week and fourth week respectively (Fig.2).

Systolic pressure of pulmonary artery was significantly elevated at the end of the study (24.8±0.74mmHg, $F=7.765$, $p=0.007$). On the other hand, the mean and diastolic pressure changes were not significant. The difgerences between groups are illustrated on Figure 3.

Pulmonary vascular and paranchymal changes of groups and statistical analysis of pathologic findings were shown in Table 3. Taken into consideration of all findings; 6 animals had G1, 1 animal had G2 and 6 animals had G3 pulmonary hypertension in experimental group, 1 had G1 and 1 had G3 pulmonary hypertension in control group (8-9). The most prominent vascular finding was arterial medial hypertrophy ($p<0.001$) and the second one was venous muscularization ($p<0.05$). On the other hand there were no meaningful ($p>0.05$) paranchymal changes (Table 3) but squamose metaplasia (n=2) and tumorlet formation (n=3) were the most interesting paranchymal findings of experimental group (Fig. 4.5).

Figure 1. Experimental desing.

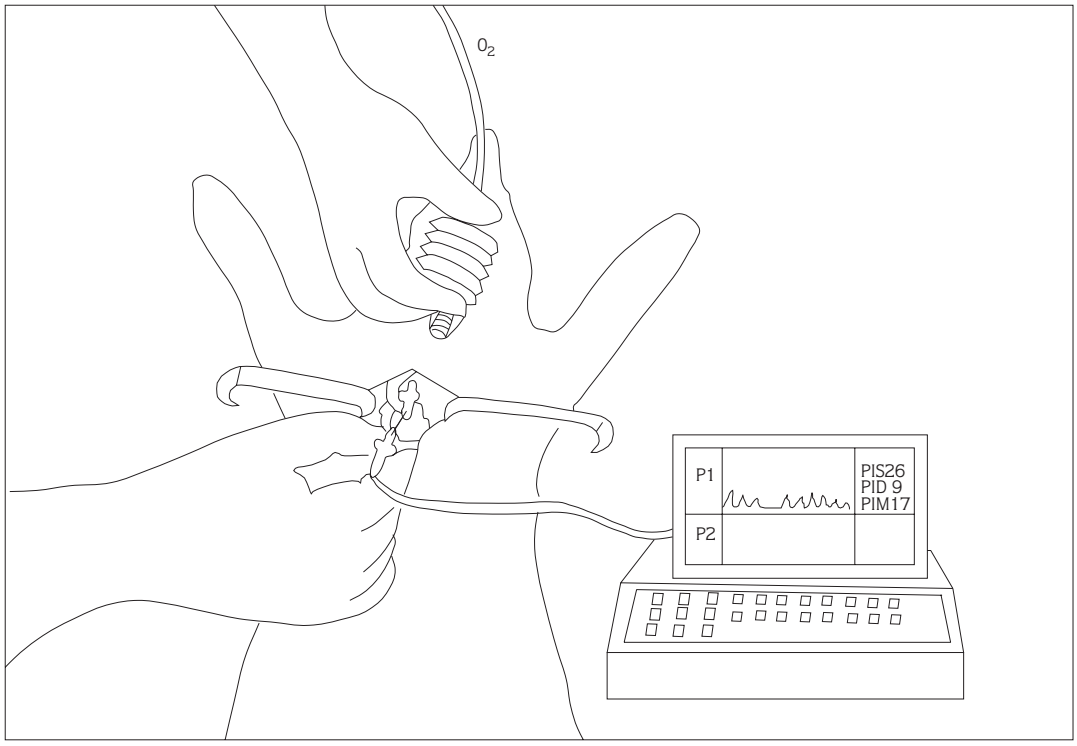

Table 2. Classification of histologic lesions for pulmonary hypertension

| |
|---|
| Group 1 (G1); Medial Hypertrophy |
| • Medial lesions: Increased smooth muscle mass, reduplication of elastic lamina, may have fibrosis in smooth muscle. |
| • Intimal lesions: Minimal intimal proliferation. |
| GROUP 2 (G2); Arteriopathy with Plexiform Lesions |
| • Medial lesions: Hypertrophied as in G1 above, may have fibrosis. |
| • Intimal lesions: "Onion skin" proliferation of cellular elements, elastic fibers and matrix in conjunction with "plexiform" lesions. |
| GROUP 3 (G3); Arteriopathy with Microthrombotic Lesions |
| • Medial lesions: Hypertrophied as in G1 above, may have fibrosis. |
| • Intimal lesions: Eccentric intimal cushions composed of cellular elements, connective tissue and matrix often in association with evidence of organized microthrombi; no plexiform lesions. |

## Discussion

Since 1981, 25 cases Primary Pulmonary Hypertension (PPH) who were treated with fenfluramine, have been reported (10-12). The mechanisms that are responsible for pulmonary hypertension in patients using fenfluramine or other amphetamine-related appetite suppressants are unclear.

Dexfenfluramine was recently taken the approval from the Food and Drug Administration for the long term treatment of obesity. There are very little experience of it's usuage in long-term.

On histopathologic examination; findings of pulmonary hypertension were encountered in all animals in the experimental group, regardless of their systolic

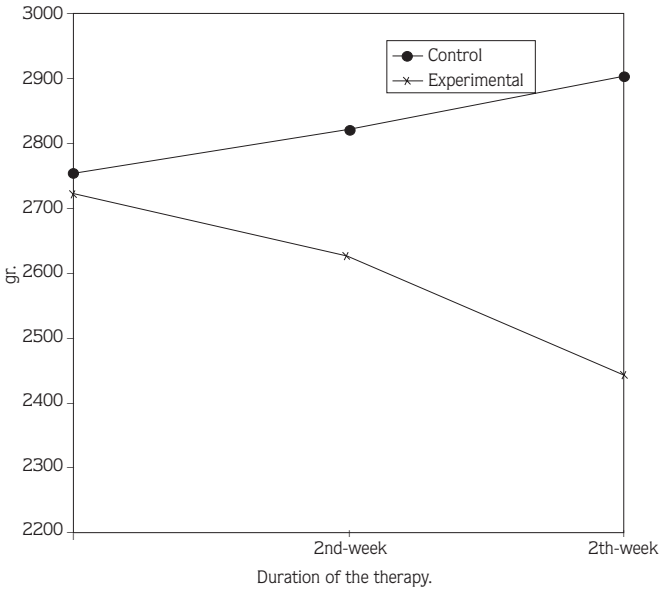


Figure 2. Body weight changes of groups. The amount of weight loss was significant ($F=8.522, P<0.01$).

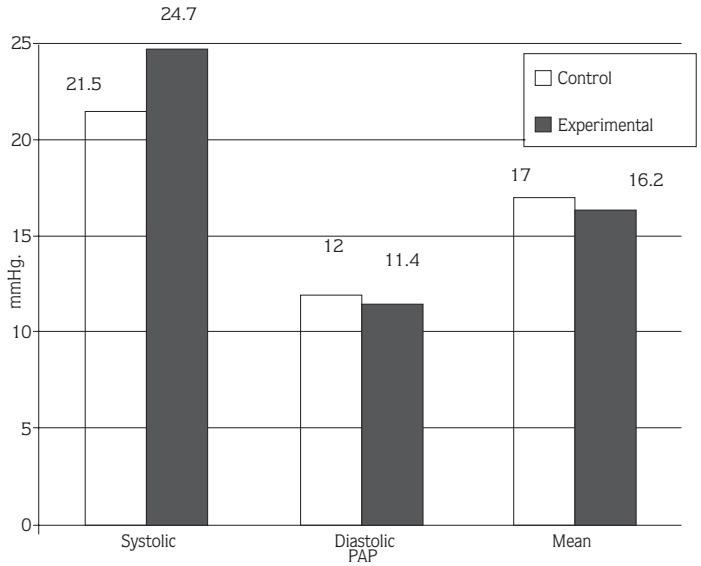


Figure 3. The comparison of PAP values of groups. Systolic pressure value was higher in experimental group (F=7.765, p<0.01)

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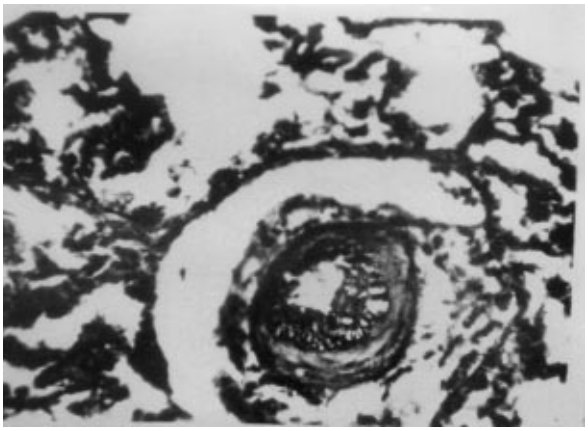
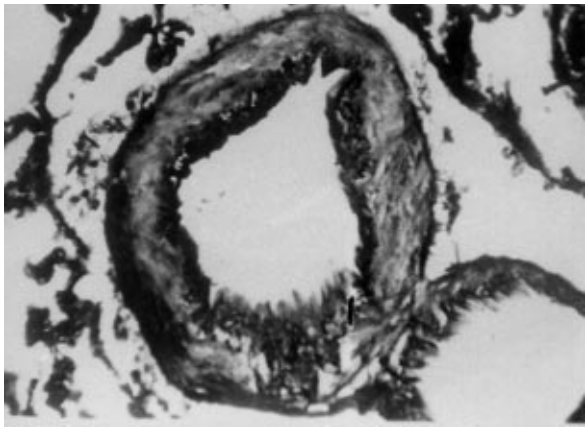
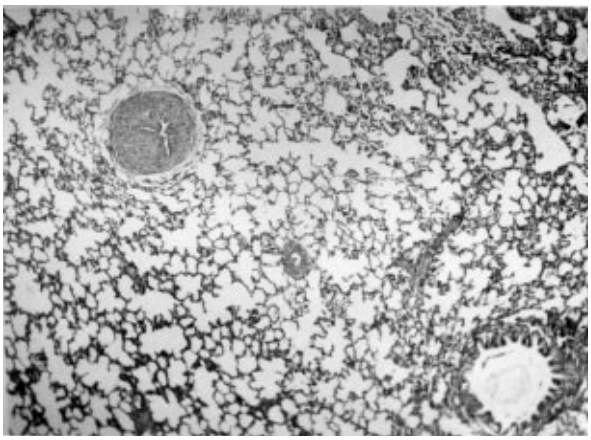


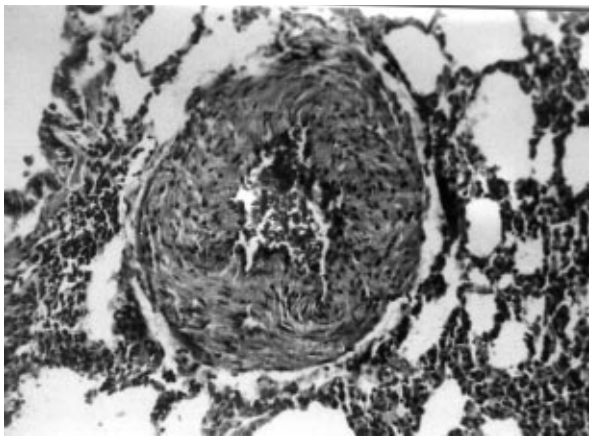
a. Repudlication of lamina elastica interna (LEI), and intimal proliferation. (Orcein Stain, x175)



b. Reduplication of LEI. (Orcein stain, x350)

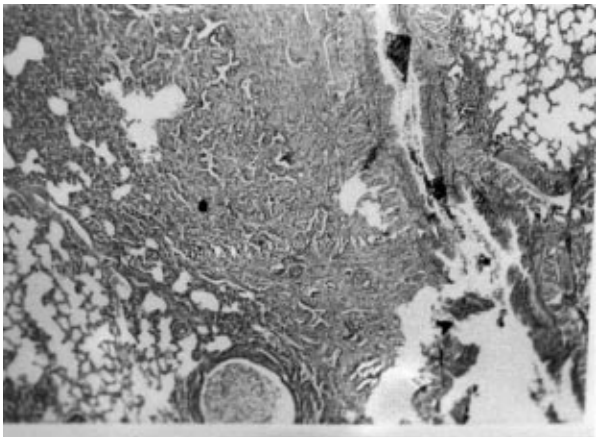


c .Medial hypertrophy (HE, x35)

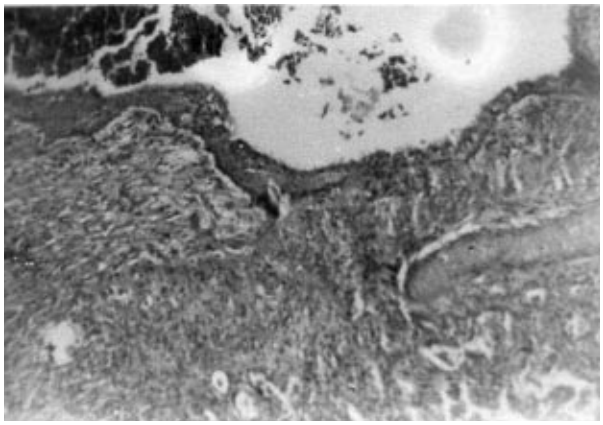


d. Medial hypertrophy (HE,x175)

Figure 4. Pulmonary vascular changes.



5a. Localize, alveolar epithelial hyperplasia (Tumorlet). (HE stain, x35)



5b. Squamose Metaplasia; bronchial epithelium. (HE stain, x90.)

Figure 5. Pulmonary paranchymal changes.

pressures. Pulmonary hypertension was detected in two animals in the control group.

In the experimental group; six animals, who had G1, one had G2 and six had G3 pulmonary hypertension. Among these animals, who had G1 hypertension, five of them had paranchymal changes due to abscess, pneumonia and fibrosis. Only one animal had pulmonary hypertension without prominent paranchymal changes, so it can be accepted as drug effect. One animal had G2 pulmonary hypertension and it was due to pneumonia. 6 animals had G3 hypertension. Three of them had additional paranchymal findings, their vascular changes that causes hypertension were due to these paranchymal changes. Whereas other three animals had vascular changes without any promoting paranchymal findigs that produce pulmonary hypertension. Since, it can be explained by the effect of the drug.

In the control group; one animal had G1 and one had G3 pulmonary hypertension. One of them had pneumonia but other one had no paranchymal changes that explains the hypertension.

As a result; no additional pulmonary paranchymal changes have seen in one G1 and three G3 pulmonary hypertension cases, so the seen vascular changes thought to be the effect of the drug.

Except three animals there were no significant paranchymal changes between groups ($p>0.05$). These three animals were in experimental group.

Marked alveolar epithelial hyperplasia (Tumorlet) was identified in their sections, one of them had squamose metaplasia (Fig. 5). These changes can be produced by directly; aspiration of gastric content due to regurgitation, or by indirectly; neoplastic effect of the drug. Both tumorlet and squamose metaplasia might be reversible. Nevertheless these kinds of lesions are potentially risk for neoplasm.

In recent studies efforts focused on clinical trials and their statistical significances rather than explanation of the pharmaco-pathologic mechanisms, since there is a little experience on animal (rabbit) investigation of this drug (4,13).

The use of appetite supressants is a risk factor for primary pulmonary hypertension, especially use lasting more than three months (4). In our study, pulmonary hypertension occured in one month. It is probably due to the dosage (7.5 mg/kg/day). Duration and dosage relation becomes important.

Weight loss was obvious ($F= 8.522$, $p<0.01$) in the experimental group. It was the expecting effect of the drug. The anorectic activity of these drugs depend on the phenylethylamine molecule that takes place in the structure. All of them have sympathomimetic and serotoninergic effects in varying degrees (14). Cardiopulmonary effects of serotonin have been well demonstrated. Serotonin contracts isolated pulmonary arteries in dogs and humans it is a direct vasoconstructor effect through potassium channel blockade (4,15,16). In addition, it has a synergic effect on vascular smooth muscle cell proliferation with platelet-derived growth factor (17). Moreover, patients with PPH have higher levels of free plasma serotoin than that of normal individuals (12).

Table 3: Statistical analysis of groups according to pulmonary vascular and paranchymal changes.

| Vascular Changes | Control Group (n=10) | | Experimental Group (n=13) | | Fischer exact test | |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| Arterial Medial Hypertrophy | 1 | 10 | 11 | 85 | p=0.0006 | p<0.001 |
| Arterial Intimal Proliferation | 1 | 10 | 6 | 46 | p=0.09 | p>0.05 |
| Thrombosis | 1 | 10 | 6 | 46 | p=0.09 | p>0.05 |
| Arterial Elastic Lamina Duplication | 0 | 0 | 3 | 23 | p=0.23 | p>0.05 |
| Venous Muscularization | 0 | 0 | 5 | 23 | p=0.046 | p<0.05 |
| Wall Thickening | 0 | 0 | 2 1 | 5 | p=0.31 | p>0.05 |
| Arterial plexiform Lesions | 0 | 0 | 1 | 8 | p=0.57 | p>0.05 |

| Paranchymal Changes | Control Group (n=10) | | Experimental Group (n=13) | | Fischer exact test | |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| Atelectasis | 4 | 40 | 4 | 30 | p=0.6 | p>0.05 |
| Emphysema | 5 | 50 | 9 | 69 | p=0.4 | p>0.0 |
| Pneumonia | 2 | 20 | 7 | 54 | p=0.19 | p>0.05 |
| Abscess | 0 | 0 | 2 | 15 | p=0.4 | p>0.05 |
| Congestion | 4 | 40 | 8 | 61 | p=0.41 | p>0.05 |
| Hemorrahge | 3 | 30 | 7 | 54 | p=0.40 | p>0.05 |
| Edema | 1 | 10 | 2 | 15 | p=0.6 | p>0.05 |
| Fibrosis | 0 | 0 | 2 | 15 | p=0.60 | p>0.05 |
| Pleuritis | 1 | 10 | 3 | 23 | p=0.60 | p>0.05 |
| Squamose Metaplasia | 0 | 0 | 2 | 15 | p=0.49 | p>0.05 |
| Tumorlet | 0 | 0 | 3 | 23 | p=0.23 | p>0.05 |

The present study shows that systolic pulmonary arterial pressures are increased with dexfenfluramine therapy ($F= 7.765$, $p<0.01$). We could not measure plasma serotonin levels due to technical difficulties. This elevation is probably due to increased level of serotonin and consequently vascular smooth muscle proliferation.

Obese persons are under at risk of various disease including primary pulmonary hypertension (1:500.000). The risk is 30 times higher for obese persons who use anorexic agents (4).

Most of obese patients prefer to use this kind of drugs for esthetic perplexities. Body health is closely

related with normal body mass. Obese patients should prefer natural ways such as; changing eating habbit and doing much more exercise.

We recommend clarifying hypertensive and neoplastic effects of this drug, before using it in long term.

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