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# DOES VITAMIN A IMPROVE IMMUNITY IN NEW BORN? A CONTROVERSY

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**Authors contribution:**
* Generation of idea of research work, its conduction, data collection, analysis and manuscript with final. ** Helped in assessing results and writing discussion. *** Helped in writing introduction, data collection and analysis.

**Conflict of interest**: Does Vitamin A improves immunity in new borns in terms of thymir popular.

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## ABSTRACT

**Objective:** To evaluate the effects of Vitamin A excess on immunity of albino rats in term of T-Lymphocytes. **Study design:** Experimental. **Place and Duration of Study:** This study was conducted in the Department of Anatomy, University Medical and Dental College, from June 2015 to June 2016. **Materials and Methods:** Study was conducted with 36 rat pups, of Sprague dawley variety. They were randomly separated into two equal groups A and B (n=18) on postnatal day 1. The synthetic form of Vitamin A was isotretinoin (13-cis retinoic acid). Therapeutic doses of isotretinoin, 2.5 mg/kg body weight was given to the pups of experimental groups by N/G tube. We selected mid-day of every week (days 4, 11, 18 and 25) in four postnatal weeks to deliver dose Vitamin A to pups. Group A was control group while group B was experimental group. On 30th postnatal day all pups were euthanized and weighed. Their thymuses were then dissected for Histological study. **Results:** As compared to the pups in the control group the mean thymocyte population of experimental group was found to decrease ($P<0.01$). The mean population of immature thymocytes of experimental group B was also found to decrease ($P<0.05$). **Conclusion:** Results of our research indicate that immunity of an albino rat pup could be decreased in terms of T-Lymphocytes, by the frequent use of therapeutic dose of vitamin A. **Recommendations:** Applying this result to human care should be taken to use vitamin A in a new born.

**Key words:** Vitamin A, T-Lymphocytes, Immunity.

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## INTRODUCTION

Vitamin A is a fat soluble vitamin.[1] It was discovered by Elmer McCollum at the University of Wisconsin-Madison in 1917. Vitamins were obtained solely through food intake until the 1900s, but afterward they have

been available as inexpensive pills for several decades, to supplementary daily diet. It can be obtained by animal foods like liver, kidney and milk. Plant sources of vitamin A includes carrots, spinach and peas, however, in plant sources it is obtained in â-carotene form. [2]

Vitamin A has different forms like: Retinol, Retinal, Retinoic Acid, Retinyl Esters and Carotenoids. The term retinoid is used for biological active form of all natural and synthetic forms of vitamin A.[3]

Vitamin A is an essential vitamin that helps regulate cellular differentiation of epithelial tissue. [4, 5] Daily requirement of vitamin A for male is 3000IU/day and for female it is 2300 IU/day.[6]

As Vitamin A is fat soluble, its elimination through the body is much slower than water soluble Vitamins B and C, so that the toxic level can be attained very easily. Excessive intake of Vitamin A produces a toxic syndrome called hypervitaminosis A. This can cause vomiting, nausea, anorexia, jaundice, weakness, drowsiness, blurry vision, headaches, muscles and abdominal pains.[7]

Vitamin A is widely used in clinical practice to treat Xerophthalmia, dermatological problems, such as acne and psoriasis and chronic infections. In many developing contraries where infectious diseases like diarrhea and measles were responsible for child mortality, vitamin A supplementation programs were launched. It was noted that both disease severity and mortality were decreases. It was assumed that vitamin A probably has some role in immune system of body.[8]

There are various studies on rats and mice which shows that excess intake of vitamin A by pregnant women could be harmful for developing fetus. There could be malformations of fetuses, faces, limbs, skulls, central nervous system and eyes . It was noted that neural crest derived organs were especially affected. Conotruncal heart defects were also reported in fetus.[9]

Many studies also show that excess vitamin A can cause decrease fetal weight.[10]

This study was therefore designed to investigate that if excess intake of vitamin A is dangerous for fetus then this could also be harmful for newborns and children.

We selected thymus in this study for two reasons. Firstly, literature review has proved that vitamin A especially affects the development of neural crest cell derivatives.[11] Secondly, we wanted to investigate the immunity in terms of thymocytes population.

## MATERIALS AND METHODS

This experimental study was conducted in Department of Anatomy, University Medical and Dental College Faisalabad from June 2015 to June 2016.

In this experimental study 12 female rats, weighing about 200-250 g and 04 adult male rats, weighing 250-300 g of Sprague-dawley variety of albino rats were used. They were obtained from National Institute of Health, Islamabad.

All animals were kept separately in animal house and were acclimatized for 15 days. They were provided with chick food and water.

### Conception

For conception three female rats and one male rat were kept together in a cage for a week.

### Study Design

Rats were allowed to be delivered normally. Three pups from each rat were selected randomly and labeled as group A and B Group A was control group and group B was experimental group. Isotretinoin (13-cis retinoic acid) in oral form was used in this study (capsule Roaccutane, Roche), given to the rats by nasogastric tube (N/G Tube).

### Group A

It was the control group containing 18 pups and they were given 1ml of olive oil through N/G Tube.

### Group B

--These pups were given 2.5mg/kg body weight of isotretinoin for four weeks on every midday of the week (days 4, 11, 18 & 25).

Pups of both groups were euthanized on 30th postnatal day. This procedure was approved by IRB (Institutional Review Board), a research ethical committee. Before dissection, these animals were weighed and their thymuses were removed for detailed morphological study.

### Collection of sample size

12 female rats were allow to delivered normally. Then 3 pups were randomly taken from each female rats (n=36). These pups were then two groups (n=18).

The sample size was calculated by using body weight of offspring. The expected difference of 1.5 g with a standard deviation of 1.6 at 0.05 level of significance. The sample size per group was 18 (3 per animal) with power test of 96.04%.

The number of rats per group was decided on the basis of average number of off springs per animal. The expected difference of 2 off springs with standard deviation of 1.4 at 0.05 level of significance. The calculated size was 6 rats per group with 80 percent power of test.

So the total sample size in this study was 18X2=36. The following parameters were observed in the study of the thymus:

**Histological Parameters**

Zenker's solution was used to fix the thymus. After fixation thymus was embedded in paraffin. Serial 5µm sections were cut and stained with hematoxylin/ eosin stains for detailed histological study of thymus. The following parameters were calculated.

1) Thymocyte population
2) Apoptosis
3) Immature thymoblasts (Mitotic figures)
4) Diameter of thymic lobules (µm)

The diameter of thymic lobules was measured with the help of ocular micrometer in µm.

**STATISTICAL ANALYSIS:**

Data was analyzed by t-test using Statistical Package for Social Sciences (SPSS). All the quantitative variables were described by Mean, ± SD. P-value of < 0.05 was considered significant.

## RESULTS

As compared to the pups in the control group the mean thymocyte population of experimental group was found to decrease. Difference was significant statistically (P<0.01). No statistically significant difference was found in mean apoptosis. The mean population of immature thymocytes of experimental group B was found to decrease. Difference was significant statistically (P<0.05). Mean diameter of thymic lobules showed no statistically significant change in experimental group. (Table)

## DISCUSSION

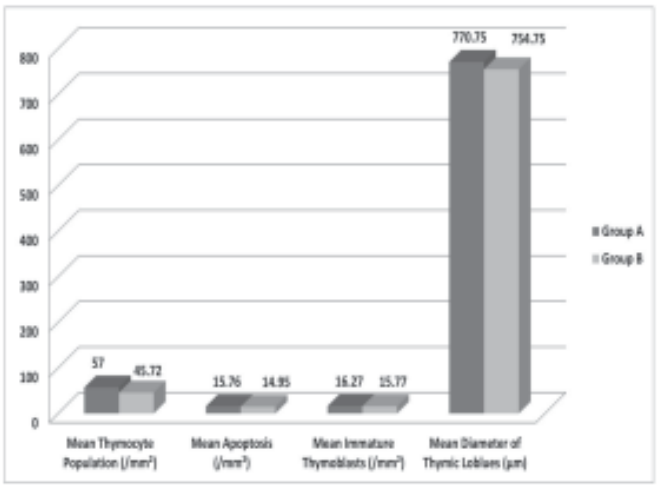
This study was designed to evaluate the effects of Therapeutic dose of vitamin A on the immunity of a 30 days old albino rat, in terms of T-Lymphocytes. Mean T-lymphocyte population was decreased significantly in experimental group. That group received 4 doses of vitamin A up till 30$^{th}$ postnatal day. So it can be concluded that frequent doses of vitamin A can cause T-lymphocyte depletion.

**TABLE**

**Effects of Vitamin A on Thymocyte Population, Apoptosis, Immature thymoblasts and Diameter of thymic lobules**

| | Group B | | | | Group A | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **S.D** | **Min.** | **Max.** | **Mean** | **S.D** | **Min.** | **Max.** | **P-value** |
| **Thymocyte Population** / mm$^2$ | 45.72 | ±0.89 | 44.00 | 47.00 | 57.00 | ±2.38 | 54.00 | 62.00 | <0.001** |
| **Apoptotic cells** / mm$^2$ | 14.95 | ±2.01 | 10.60 | 16.80 | 15.76 | ±0.50 | 14.50 | 16.50 | 0.115++ |
| **Immature thymoblasts** / mm$^2$ | 15.77 | ±0.744 | 14.40 | 16.90 | 16.27 | ±0.45 | 15.20 | 16.90 | 0.023* |
| **Diameter of thymic** | | | | | | | | | |
| **loblues (um)** | 754.75 | ±3.35 | 678 | 836.25 | 770.75 | ±1.55 | 687.5 | 850 | 0.070++ |

A Control Group
B Experimental Group
S.D Standard Deviation
Min. Minimum
Max. Maximum
**Highly significant difference(P<0.01)
* Significant difference (P<0.05)
++ Non significant difference (P>0.05)
Based on t-test




Effects of Vitamin A on Thymocyte Population, Apoptosis, Immature thymoblasts and Diameter of thymic lobules

In experimental group mean population of immature T-Lymphocytes was also found to be decreased significantly. These are the immature T-Lymphocytes, which grow to form mature T-Lymphocyte. So, less number of immature T-Lymphocytes may predict that there will be a decrease in mean T-Lymphocyte population. Literature showed that all-trans retinoic acid could inhibit T-Helper cells1 (TH1) development while simultaneously promoting T-helper cells2 (TH2) development in vitro. [12, 13]

In another study 9-cis retinoic acid also claimed to suppress TH1 development.[14]

Our study had limitation of H&E staining by which we could not differentiate between TH1 and TH2 cells. So we simply counted the number of T-Lymphocyte. Apoptosis is a programmed cell death.

It had proved that metabolites of vitamin A increased the apoptosis in T-Lymphocytes.

For this property RA analogues have been reported to play an important role in adjuvant therapy in cancers.[15]

But in our study no significant change in apoptosis was noted. This could be due to as using therapeutic dose of vitamin A in this study. Higher doses of vitamin A might cause increased apoptosis in Thymus but that was not part of our experiment.

This study suggested that vitamin A can decreases the population of T-Lymphocyte in body. As we know T-Lymphocyte are the part of innate immunity. So the missing part of immunity could invite infections in a new born.

## CONCLUSION

Results of our research indicate that immunity of an albino rat pup could be decreased in terms of T-Lymphocytes, by the frequent use of therapeutic dose of vitamin A.

## RECOMMENDATIONS

Applying this result to human care should be taken to use vitamin A in a new born.

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