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### Original Article

#### A Comparative Study of Cholesterol Levels in Rabbits fed with Oral Contraceptives and in Combination with Vitamin E

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#### ABSTRACT:

**Introduction :** Cholesterol metabolism is influenced by many factors such as OCP's having ethinyl estradiol along with levonorgestrel. These hormones influence cholesterol metabolism and its protective effect with supplementing Vitamin E was studied on rabbits. **Aims & Objectives:** The cholesterol levels were compared on the first day of study and on completion of 90 days of study on rabbits in all the 3 groups. In group A rabbits were fed only on stock diet. In group B the rabbits were fed on stock diet along with OCP's and in Group C the rabbits were fed on stock diet along with OCP's plus vitamin E and the changes in levels of serum cholesterol were recorded. **Materials & Methods :** The present study was conducted in the Department of Physiology Govt. Medical College, Amritsar and in collaboration with the Department of Physiology Chintpurni Medical College & Hospital, Bungal, Pathankot (CMC & H, Bungal, Patnankot). The practical work was done on rabbits at Govt. Medical College, Amritsar (G.M.C Amritsar) and study is being published at Chintpurni Medical College & Hospital, Bungal, Pathankot. **Results:** The cholesterol levels increased on addition of OCP's to the stock diet but the levels were decreased on the addition of supplements like Vitamin E. **Conclusions:** The addition of OCP's disturb lipid metabolism but it can be overcome by addition of supplements like Vitamin E.

**Key words:** OCP's (Oral Contraceptive Pills), HDL-C (High Density Lipoprotein Cholesterol)

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#### INTRODUCTION

Population explosion is a global phenomenon among the various developing and developed countries. India is one of the most thickly populous country. To control this, various methods of contraception are being employed of which, combined oral contraceptive are almost 100 % effective in preventing pregnancy.

Among the developed countries of the world, contraceptive pills containing varying amount of progestogens and oestrogens are being very commonly used. In India, too this method of contraception is getting very popular as its efficacy is definitely more than other contraceptive measures.

Contraceptive pills, though very effective in controlling population also have severe adverse effects associated with

their prolonged use especially thromboembolic episodes leading to myocardial infarction and cerebral strokes.

Steroids present in oral contraceptives are known to cause metabolic changes especially alteration in the lipid metabolism which in turn have been labelled to be responsible for the increased risk of thromboembolic episodes. It is observed that hypercholesterolemia and hypertriglyceridemia are risk factors for the development of coronary heart disease. They operate independently of each other and a combined effect of cholesterol and triglycerides carries high risk for the development of ischaemic heart disease.

The adverse effects are especially known to be associated with high dose of combined oral contraceptive use and therefore, more and more attempts have been made to bring

out new products with reduced doses of steroid components<sup>[1]</sup>. Oral contraceptives have been known to decrease HDLC (High Density Lipoprotein cholesterol) and HDLC is believed to be inversely related to the incidence of myocardial infarction. So many efforts had been made to find the agents which could increase the HDLC levels and thus in turn provide protection against thrombotic episodes leading to myocardial infarction and increased incidence of cerebral strokes<sup>[1]</sup>.

Various serum cholesterol reducing and HDLC increasing agents so far tried are garlics, vitamin E, Physical exercise which has one or more short coming<sup>[1]</sup>.

Recently, researchers have revised the interest of use of Vitamin E as serum HDLC increasing agent as it is an antioxidant<sup>[1]</sup>. There are also some studies which have observed the effect of vitamin E in a study on human beings and on rabbits<sup>[2]</sup>.

Since any drug if used for a long period by a fairly large section of population must be studied with close scrutiny as to its hazards which may be immediate or delayed<sup>[3,4]</sup>. The variable effects of oral contraceptives on the serum cholesterol and consequent effect of Vitamin E in rabbits is studied by different combinations of combined OCP's (Oral Contraceptive Pills)<sup>[5]</sup>.

Vitamin E was discovered by Evans and Bishop in 1922 in studies of fertility on female rats. Deficient animals were found to ovulate and conceive normally but at some time during the period of gestation deaths and abortion of foetus occurred<sup>[6]</sup>. In 1938 it was identified chemically by Fernholtz<sup>[7]</sup> and in the same year it was synthesized by Karrer and associates<sup>[8]</sup>. On exposure to air or Ultraviolet light it deteriorated slowly. Alpha tocopherol is the most important of tocopherols and is considered to be vitamin E and it constitutes 90 % of the tocopherols in animal tissues<sup>[9,10]</sup>. Sources include eggs, muscle, wheat, liver, fish and chicken<sup>[11,12]</sup>. Wheat germ oil, cotton seed oil & corn oil are three main sources of natural tocopherol<sup>[13]</sup>.

Human milk on an average contains 1.14 mg alpha tocopherol/ quart whether frozen, fresh or pasteurized or lyophilized whereas cow's milk contains 0.21-1.06 mg/quart<sup>[14]</sup>.

The peculiarity of tocopherols amongst Vitamins is that substantial loss of tocopherols occur during freeze storage<sup>[15]</sup>.

Vitamin E plays a role of antioxidant in many biochemical systems and has been attributed special significance in the maintenance of double bonds (unsaturation) in lipids. This function may contribute to the effect of tocopherol on serum cholesterol levels<sup>[16]</sup>.

## MATERIALS & METHODS

This study was conducted during the years 1991- 1993 in the Department of Physiology, Government Medical College, Amritsar on rabbits fed with normal diet and in combination with oral contraceptive pills (OCP's) and

Vitamin E. The rabbits were divided into three groups (A,B,C). Group-A contains rabbits fed on normal diet (control group). Group-B consisted of rabbits fed with normal diet along with OCP's, ovral-I(wyeth) containing 30µg of ethinyl estradiol & 150 µg levonorgestrel daily for 3 months and Group-C consisted of rabbits fed with normal diet along with, OCP's, ovral-I(wyeth), 30µg of ethinyl estradiol & 150 µg levonorgestrel and 7 mg of Vitamin-E daily for 3 months. The study started from day 1 and continued upto total period of three months. Blood sample was collected for serum cholesterol, after interval of every 15 days for 3 months.

## Estimation of serum cholesterol:

(Zlatkis as modified by Zak,1953)<sup>[17]</sup>

**Principle:** Serum cholesterol was extracted quantitatively into an acetone-ethanol mixture which also precipitates the proteins. The extract was evaporated to dryness and residue was dissolved in glacial acetic acid and ferric chloride colour reaction is carried out.

## Reagents:

1. Acetone –ethanol mixture (1:1 v/v)
2. Glacial acetic acid (Aldehyde free).
3. Concentrated sulphuric acid (A.R)
4. Standard cholesterol solution (200 mg%) 200mg of cholesterol is dissolved in 100ml of glacial acetic acid.
5. Ferric chloride solution(10%) 10 gm of ferric chloride 6H<sub>2</sub>O was dissolved in 100ml aldehyde free glacial acetic acid.
6. King's reagent (color developing reagent). 9.9ml of concentrated sulphuric acid was taken in a dry 100ml cylinder and 0.1ml of ferric chloride solution was added to it and mixed by shaking(prepared fresh each time).

## RESULTS

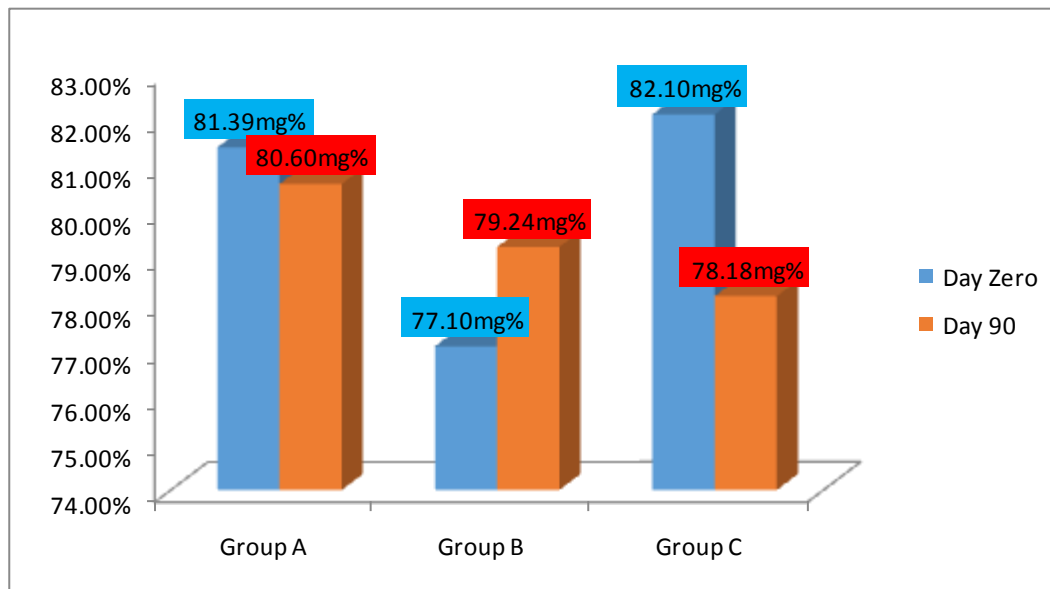
**1. Serum Cholesterol in Group A-** At zero day the mean serum cholesterol levels varied between 75.0 - 89.2 mg% with the mean level of 81.39 mg% is compared to mean serum cholesterol of 80.67 mg% on the 90 th day of study. The mean levels at the 15, 30, 45, 60, 75, 90 days were 80.66 mg%. 81.03 mg%, 81.02 mg%, 80.66 mg%, 80.69 mg % and 80.67 mg%. Thus there was no significant change in serum cholesterol levels after 90 days in rabbits getting stock diet only( $t=0.286$  and  $p>0.7$ ).

**2. Serum cholesterol in Group-B-** The mean levels of serum cholesterol with their variations at different days were as follows. Mean cholesterol levels varied between 77.10 - 79.24 mg %. The mean levels with their variations at 15, 30, 45, 60, 75 and 90 days were 77.46 mg%, 78.89 mg%, 78.90 mg%, 79.25 mg%, 79.24 mg % and 79.24 mg %. It is apparent that there was marked increase in serum cholesterol level with combined OCP's plus stock diet

administration, however the rise was statistically insignificant ( $t=0.604$  and  $p>0.5$ ) indicating that low dose OCP's did not have significant effect on serum cholesterol at the end of 90 days of study.

**3. Serum cholesterol in Group-C-** The average serum cholesterol was 82.10 mg% varied from 71.4 - 89.2 mg% which gradually decreased during the next three months of feeding of OCP's (30 $\mu$ g of ethinyl-estradiol and 150 $\mu$ g of levonorgestrel) in low dose plus 7 mg of vitamin E plus stock diet.

The mean levels with their variations after 15, 30, 45, 60, 75 and 90 days of feeding were 79.98 mg %, 79.60 mg%, 79.61 mg%, 78.54 mg %, 78.18 mg % and 78.18 mg %. It is apparent that there was gradual decrease of serum cholesterol level from the initial level of 82.10 mg % at zero day to 78.18 mg% at the end of 90 days i.e. with decrease of 3.92 mg % but the decrease was statistically insignificant ( $t$ -test = 1.571 and  $p > 0.1$ ).



**Figure 1:** Depicts Cholesterol levels in three different groups.

**Group A-** Stock(normal) diet, **Group B-**normal diet along with OCP's, ovral-L(wyeth), 30  $\mu$ g of ethinyl estradiol & 150  $\mu$ g levonorgestrel daily for 3 months, **Group C-**30 $\mu$ g of ethinyl-estradiol and 150  $\mu$ g of levonorgestrel (low dose) plus 7 mg of vitamin E plus stock diet.

## DISCUSSION

There were three study groups which were named as Group A, Group B and Group C. At that time ethical clearance was not required and the study was conducted in GMC Amritsar under the supervision and guidance of Faculty of Physiology department (Dr.Prem Khetarpal and Dr. K.C. Manchanda). This study was done during the years 1991-1993 but is being published now.

In Group A the rabbits were fed on stock (normal) diet. The cholesterol levels were recorded on day 1 and on completion of 90 days. The cholesterol levels were found to be 81.39 mg % on day 1 and on completion of 90 days, the cholesterol levels were found to be 80.60 mg %. There were no significant changes in serum cholesterol levels in rabbits on completion of 90 days who were getting stock diet only ( $p>0.70$  and  $t$ -test = 0.286). there were no significant changes since rabbits were fed on stock diet only and it does not have any statistical effect on cholesterol metabolism.

In Group B the rabbits were fed on stock diet along with OCP's (trade name ovral-L(wyeth) having 30  $\mu$ g ethinyl estradiol plus 150  $\mu$ g levonorgestrel for a period of 90 days. The mean cholesterol levels on first day was 77.10 mg % and after completion of 90 days were found to be 79.24 mg % ( $p>0.5$  and  $t$ -test = 0.604) which indicates that lower dose of oral pills did not have significant effect on serum cholesterol levels at the end of 90 days. No doubt the levels were increased due to metabolic changes in cholesterol metabolism but the increase was not found to be significantly high. It indicated that ethinyl estradiol plus levonorgestrel (OCP's) might have had some impact on lipid metabolism levels (serum cholesterol).

In Group C the rabbits were fed on stock diet along with OCP's (30  $\mu$ g of ethinyl estradiol plus 150  $\mu$ g of levonorgestrel) low dose plus 7 mg of Vitamin E. The mean cholesterol levels on first day of study was 82.10 mg % and on completion of 90 days 78.18 mg % ( $p>0.1$  and  $t$ -test=1.571) with the decrease in levels of serum cholesterol was statistically insignificant. In this group

along with OCP's (ethinyl estradiol plus levonorgestrel) along with addition of Vitamin E that had lead to decrease in metabolic disturbance causing decrease in cholesterol levels.

**CONCLUSIONS:** Cholesterol metabolism is influenced by addition of OCP's in the form of ethinyl estradiol plus levonorgestrel in the stock diet and it can be tracked down on addition of supplements like Vitamin E.

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