

# EFFECT OF UNRIPE GRAPE JUICE (VERJUICE) ON PLASMA LIPID LEVELS IN RABBITS RENDERED HYPERCHOLESTEROLEMIC BY FEEDING EGG YOLK

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**Abstract-** Since many years it has been a general belief in Iranian traditional medicine that unripe grape juice (verjuice) has lipid-lowering effect. This study was designed to test this hypothesis. Fifty rabbits were selected and divided into 5 groups with 10 rabbits in each. Group 1 had no supplemental diet. Group 2 were fed 10 ml egg yolk daily and group 3 were fed 10 ml egg yolk plus 20 ml verjuice daily for six weeks. In the second part of study, 20 rabbits rendered hypercholesterolemic by feeding egg yolk for six weeks, then they were divided into two groups: Group 4 received 10 ml of the egg yolk daily, and group 5 received 10 ml of the egg yolk plus 20 ml verjuice daily for the next 6 weeks. The plasma lipid profiles were measured at the beginning and then every two weeks. In the first part of study total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations in group 2 rose 10 times in comparison with group 1, but addition of verjuice in group 3 did not prevent rising of these values. In the second part of study, TC and LDL-C values rose in groups 4 and 5 in a parallel fashion. Changes in high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were not statistically significant throughout the study. In conclusion, this study did not support preventive or therapeutic effect of verjuice in hypercholesterolemia.

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**Key words:** Hypercholesterolemia, unripe grape juice, Verjuice, egg yolk, lipid

## INTRODUCTION

For many years, epidemiological evidence has been accumulating indicating a link among high LDL-C (low density lipoprotein cholesterol), low HDL-C (high density lipoprotein cholesterol), and coronary heart disease. Recently, solid support for

the causal association of this link has been provided by large well-conducted trials showing that lowering LDL-C, both in patients with no history of angina pectoris (primary prevention) (1), and in patients with established coronary heart disease (secondary prevention) (2, 3), reduces the incidence of acute coronary syndrome including coronary death.

For many years, it has been general belief in our society that certain foods and herbal drugs have lipid lowering effect and several studies also confirmed the efficacy of some of them (4). Unripe grape juice (verjuice) is one of these agents that according to

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Iranian folk medicine have lipid-lowering effect (5).

The aim of this study was to evaluate the preventive and therapeutic effects of verjuice in hypercholesterolemia.

## MATERIALS AND METHODS

Fifty young female New Zealand white rabbits in the initial weight range of 1500-2000 grams (obtained from animal laboratory of Shiraz Medical School, Shiraz, Iran) were divided randomly into five groups with 10 rabbits in each. Animals were housed in individual metal cages in a temperature-controlled room. Commercial rabbit diets (Pars Dam Company, Tehran, Iran) in the form of pellets and tap water were available for all rabbits throughout the experiment *ad libitum*. Eggs and verjuice were purchased from the local markets. All experiments on animals were performed in accordance with UK legal requirements.

The experiment was designed in two steps. The objects of step I, which was conducted for six weeks, were evaluation of the effect of egg yolk as a hypercholesterolemic diet on plasma lipid levels and also the effect of verjuice in prevention of the development of hypercholesterolemia in rabbits, which were fed hypercholesterolemic diet. The aim of step II, which was conducted for twelve weeks, was evaluation of the therapeutic effects of verjuice in hypercholesterolemic rabbits. Step I consisted of three and step II of two groups of rabbits. Group I received no supplemental food for six weeks; Group II received 10 ml egg yolk daily for six weeks and Group III received 10 ml egg yolk plus 20 ml verjuice daily for six weeks. Rabbits of group IV and V were rendered hypercholesterolemic by feeding egg yolk for six weeks and then they received 10 ml egg yolk daily for the next six weeks (Group IV) or 10 ml egg yolk plus 20 ml verjuice daily for the next six weeks (Group V).

The yolk of eggs were separated manually and pooled in a clean container and mixed. Then 10 ml of mixed yolk was administered through a clean 16 gauge sound catheter placed in the stomach of the animals. After 15 minutes, 20 ml unstrained verjuice

was also administered in selected groups (III and V) by similar procedure. At the beginning of experiment and every two weeks up to the end of experiment, rabbits, after 14-16 hours of fasting, were anesthetized by intramuscular injection of 1.5 ml ketamine and 0.5 ml xylazine and then 2-3 ml blood were drawn by insertion of heparinized 23 gauge butterfly intravenous catheter into central ear artery. A 100-watt light bulb as a source of heat and locally applied nitroglycerin ointment over the auricular artery were used to dilate the vessels. The plasma was immediately separated by centrifugation at 5000 rpm for 15 minutes at room temperature. Total cholesterol (TC), LDL-C, HDL-C and triglyceride (TG) concentrations were determined after storage of samples at  $-70^{\circ}\text{C}$  for several weeks by the commercially appropriate kits (Randox Company, UK).

At the end of experiment, anesthetized rabbits were killed by a blow on the head. The aorta was removed, rinsed with water and preserved in 10% formalin solution for histological examination. After preparation and staining with hematoxylin and eosin (H&E), specimens were examined by a pathologist who was unaware of details of groups with light microscopy.

Results are presented as means of 10 animals  $\pm$  standard errors of means ( $M \pm \text{SEM}$ ). Statistical significance of differences between values were determined by one way ANOVA with Duncan procedure for data in step I and paired *t* test for data in step II of experiment. In all cases the probability of type 1 error  $< 0.05$  was taken as the criterion of significance. All statistical analyses were conducted using SPSS version 9 computer program.

## RESULTS

Plasma lipid levels (mg/dl) in various groups during the step one are shown in the tables 1 and table 2 shows plasma lipid levels (mg/dl) in various groups during the step two of experiment.

Step I- The egg yolk had a profound effect on TC and LDL-C. These two parameters rose to 10 times of the baseline values at sixth week, but observed

**Effect of verjuice on plasma lipids**

**Table 1.** Plasma lipid levels (mg/dl) in various groups during the step one of experiment\*

Groups	TC		LDL-C		HDL-C		TG	
	0	6 wks	0	6 wks	0	6 wks	0	6 wks
Group 1 (control)	85 ± 4	84 ± 5	40 ± 3	46 ± 2	32 ± 1	32 ± 2	45 ± 6	64 ± 8
Group 2 (6 wks egg)	86 ± 9	651 ± 80	42 ± 9	421 ± 62	30 ± 2	40 ± 5	55 ± 8	70 ± 15
Level of significance (group 2 versus 1)	<i>P</i> > 0.05	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> < 0.001	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05
Group 3 (6 wks egg + verjuice)	90 ± 14	680 ± 68	60 ± 8	458 ± 76	31 ± 1	52 ± 10	52 ± 5	69 ± 9
Level of significance (group 3 versus 2)	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05

Abbreviations: TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; wks, weeks.

\* Data are mean ± SEM of 10 rabbits in each group.

changes in HDL-C and TG levels were minimal and were statistically insignificant (group II versus I). As shown in table 1, the addition of verjuice did not prevent the rising of TC and LDL-C caused by feeding egg yolk. Changes in HDL-C and TG levels were also not significant (group III versus II). There was no atheromatous plaque in histological examination of aorta sections of all rabbits at the end of step I of experiment.

Step II- As expressed in the table 2, six weeks after egg yolk administration, there was no statistically significant differences in the amount of TC, LDL-C, HDL-C, and TG between groups IV and V.

During the next six weeks, total cholesterol and LDL-C values in groups IV and V rose in a parallel fashion. Again, the changes in HDL-C and triglyceride were not statistically significant (group V versus IV). There was also no aortic atheromatous plaque in any of the rabbits at the end of this step.

**DISCUSSION**

In this study, rabbits were rendered hypercholesterolemic by feeding egg yolk, by the method that was presented first by Srilatha *et al.* (6). Because the commercial hypercholesterolemic diet is not readily available in Iran, this may become a practical animal model for induction of hypercholesterolemia. According to our results, verjuice neither decreases TC, LDL-C and TG, nor increases HDL-C levels, in both prevention (step I) and management (step II) of hypercholesterolemia. At the end of both steps, no atheromatous plaque was observed. This is likely because atherosclerosis is a chronic process and requires more time for development. Walker *et al.* showed that in the first 12 weeks of feeding a cholesterol-rich diet, rabbit aortas were covered with an intact endothelium (7).

Focal areas of increased endothelial cell and smooth muscle cell replication were observed after

**Table 2.** Plasma Lipid Levels (mg/dl) in various groups during the step two of experiment\*

Groups	TC		LDL-C		HDL-C		TG	
	6 wks	12 wks	6 wks	12 wks	6 wks	12 wks	6 wks	12 wks
Group 4	645 ± 76	1083 ± 66	435 ± 46	700 ± 64	53 ± 7	44 ± 8	75 ± 10	101 ± 31
Group 5	620 ± 61	1009 ± 80	403 ± 44	674 ± 70	44 ± 4	42 ± 5	68 ± 12	94 ± 21
Level of significance	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05

Abbreviations: TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, wks: weeks

\*Data are given as mean ± SEM of 10 rabbits in each group

12 and 20 weeks of feeding of the lipid-rich diet, respectively. In literatures, there is no study about unripe grape juice (verjuice), but there are a few studies about the effects of grape juice in atherosclerotic cardiovascular diseases. Goldberg *et al.* conducted a study in which subjects were randomly allocated to four groups (8). Group I and II consumed 375 ml per day of red or white wine, respectively. Group III and IV received 500 ml per day of high and low phenols content grape juices for periods of 4 weeks. They observed that grape juices of either type had virtually no effect on serum lipid levels but both red and white wines raised HDL-C, apo A-I and apo A-II concentrations as well as apo A-I: apo A-II ratio to a similar extent. They concluded that favorable effects of wines in modulating plasma lipid and lipoprotein concentrations are probably due to their alcohol content and cannot be reproduced by grape juice. Despite the lack of effect of grape juice on lowering the serum lipid levels in above studies, a few other studies showed favorable effect of grape juice against atherosclerotic cardiovascular disease by several other mechanisms including modulating platelet function (9-13), enhancement of nitric oxide release (13), improvement of endothelial function (14, 15) and reduction the susceptibility of LDL-C to oxidation, an effect which should be attributed to the presence of polyphenolic compounds as an antioxidant substance in grape juice (15-19). Although we did not observe the hypocholesterolemic effect of verjuice, but it may inhibit atherosclerosis by mechanisms other than modifying lipid levels as proven for grape juice (9-19).

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### Conflict of interests

We have no conflict of interests.

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### Effect of verjuice on plasma lipids

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