

A COMPARATIVE STUDY ON EFFECT OF LEMON JUICE WITH LEMON SEEDS VS. LEMON JUICE ALONE ON HIGH SENSITIVITY C-REACTIVE PROTEIN IN SUBJECTS WITH OBESITY UNDERGOING CALORIE RESTRICTION- A PILOT STUDY

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ABSTRACT

BACKGROUND

Obesity or overweight is the most common problem faced by millions of people in this world today. Obesity is a chronic inflammatory lifestyle related disorder that increases the preponderance for many illnesses like coronary heart disease, hypertension, diabetes, cancer etc. Calorie restriction has been shown to reduce weight in obese individuals and confer health benefits. Triterpenoids have hypolipidaemic, anti-obesity, anti-hyperglycaemic and anti-cancerous effect.

In this study, we will evaluate the use of lemon seeds rich in triterpenoids on inflammatory markers in subjects with obesity undergoing calorie restriction.

MATERIALS AND METHODS

This randomised controlled study was conducted on 30 subjects of age between 20 - 60 years for a period of 7 days; were studied by dividing them into two groups; 15 subjects each in intervention and control group respectively.

RESULTS

All the parameters were compared between 2 groups. After giving lemon seeds Hs-CRP, TG, VLDL, BMI, Weight, WC, HC and WHR had decreased when compared to control group. HDL levels had reduced in both groups.

CONCLUSION

This study states that lemon seeds extraction, which is rich in triterpenoids is more effective than lemon juice which is rich in vitamin-C.

KEYWORDS

High Sensitive C-Reactive Protein, Body Mass Index, Waist Circumference, Hip Circumference, Waist-to-Hip Ratio.

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BACKGROUND

Obesity or overweight is the most common problem faced by millions of people in this World today.¹ It is a complex multifactorial condition, in which excess body fat may put a person at health risk.² Increasing evidence suggests that obesity is not a simple problem of will power or self-control, but a complex disorder involving appetite regulation and energy metabolism that is associated with a variety of comorbid conditions.³⁻⁶ Obesity is a chronic inflammatory lifestyle related disorder that increases the preponderance for many illnesses like coronary heart disease, hypertension, diabetes, cancer etc.

High-sensitivity C-reactive protein (Hs-CRP) levels are closely associated with obesity.⁷ Obesity and insulin resistances portend a significant risk of atherosclerotic disease.⁸⁻¹¹ Elevated triglycerides, moderate elevations in small dense LDL cholesterol and decreased HDL cholesterol define the atherogenic dyslipidaemia (combined hyperlipidaemia) of insulin resistance.¹¹⁻¹⁴

Calorie restriction has been shown to reduce weight in obese individuals and confer health benefits. In caloric restriction, inflammatory status is decreased by low energy intake that results in less energy supply to immune cells to favour energy saving under calorie restriction.¹⁵ Lemon seeds are rich in triterpenoids¹⁶ and they have hypolipidaemic, anti-obesity, anti-hyperglycaemic and anti-cancerous effect.^{17,18-21} Naturally occurring limonoids in citrus fruits have anti-obesity and anti-hyperglycaemia effects.¹⁸ Supplementation of naturally occurring vitamin C in higher concentrations does have effect on serum cholesterol levels, that they reduce LDL levels.¹⁹ The triterpenoids that are present in lemon seeds/ juice have anti-hyperglycaemic and hypolipidaemic effects.^{20,21} Citrus flavonoids and tocotrienols that are present in lemon have effect on serum cholesterol,

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i.e. they reduce blood levels of cholesterol.¹⁷ It is clinically proven that consumption of diet rich in fruits, vegetables and other naturally derived constituents is associated with lower incidences of chronic diseases,²² oxidative stress²³ and reduced inflammatory levels.²⁴ Hence, in the current proposal we were interested to understand the benefit of triterpenoids rich diet in reducing inflammation and cholesterol levels in obese subjects.

MATERIALS AND METHODS

Objectives

To evaluate the effects of intake of lemon juice with lemon seeds extract during calorie restriction on high sensitivity C-reactive protein [Hs-CRP] obese subjects.

Subjects

30 subjects of age between 20 - 60 years were participated in this study. They were screened through a routine medical check-up and those satisfying diagnostic criteria for obesity were recruited in this study.

Selection Criteria for Study

Inclusion Criteria

1. Age 20-60 yrs.
2. Obese individual with BMI > 25 < 35.
3. History of hypercholesterolaemia or dyslipidaemia.

Sample Size (n= 30)

Sample size is taken as per our convenience.

Group-1 (n= 15)

Received calorie restricted diet with lemon juice + lemon seeds extraction.

Group-2 (n= 15)

Received calorie restricted diet with lemon juice without lemon seeds extraction.

Study Design

Randomised controlled study.

Randomisation

Patients were randomised using computerised randomisation table and were allocated into two groups of 15 each.

Study Intervention Chart

Total 7 days study plan for each subject in this present study:
- Lemon juice with lemon seeds extraction was given only for 5 days and 2 doses had been given in a day (between each dose of 10 - 12 hrs. gap had been given) and last 2 days only lemon juice were given according to this study.

Totally, 500 mgs of lemon seeds extraction was given in a day, 250 mgs - 0 - 250 mgs.

Making Lemon Juice

For one glass of lemon juice: 300 mL of water + 2 teaspoons of lemon juice + 1/2 teaspoon of jaggery are mixed and extra 250 mgs of lemon seeds extraction was added.

Time of Giving Juices

8 am - 300 mL of lemon juice with 250 mgs of lemon seeds extraction.

10 am - only 300 mL of lemon juice.

12 am - 300 mL of lemon juice.

2 pm - 300 mL of lemon juice.

4 pm - 300 mL of lemon juice.

7:30 pm - 300 mL of lemon juice with 250 mgs of lemon seeds extraction.

Starting 5 days (1st - 5th day) all the subjects are on calorie restricted diet like 1 time fruit diet (like 1 small piece of water melon/ 1 small piece of papaya) was given with lemon juice with lemon seeds extraction. Totally, 6 glasses of lemon juice were given in a day (2 glasses with lemon seeds extraction + 4 glass of only lemon juice) 3 - 4 litres of water is taken by each subject. Last 2 days (6th - 7th day), 2 times fruits diet (like 1 small piece of water melon, 1 small piece of papaya) + only lemon juice without extraction.

Steps in Extraction of Lemon Seeds Extraction

The lemon seeds are dried in sunlight and powdered by using grinder and extraction unit 2046 and control unit 2046 are used to make lemon seeds extraction and 50 - 70 mL of the solvent-ethanol alcohol as be used. After all the following steps, extraction was taken in Petri dish and allowed it to dry for 2 - 3 days.



Lemons and Lemon Seeds Extraction

The Extraction Unit 2045 and the Control Unit 2046

In the extraction unit, solvent extraction was done in two steps. At first, the sample was immersed in the boiling solvent to dissolve most of the soluble material. In the second step, the sample was raised about the solvent surface to permit an efficient washing of the sample with solvent from the condensers.²⁵

After extraction, the condenser valves were closed by turning the valve knobs a quarter turn. After some minutes most of the solvent is collected in the condenser. The last residue of solvent was evaporated when the air valves were opened.²⁵

The control unit controls the temperature of the hot plate. It has timer function for the various extraction procedure steps.²⁵



The Extraction Unit 2045 and the Control Unit 2046

Methods

Inflammation is measured by Hs-CRP. All the parameters like Hs-CRP and Anthropometry measurements were taken pre (1st day) and post (8th day) of the intervention.

Blood was drawn from an antecubital vein in the morning after a 12-hour overnight fasting and was sent for analysis within four hours of blood collection. Hs-CRP levels were measured by immunoturbidimetry method by a biochemical autoanalyser at the Central Laboratory, Bengaluru.

Anthropometric measurements were obtained from the complete physical examination. Weight and height were measured with the subject's shoeless and wearing light clothing. BMI was derived from the formula, weight (kg) ÷ (height) 2 (m2). With the participant standing, WC was measured midway between the superior iliac crest and the costal margin and hip circumference at its maximum protrusion point of the buttocks around the pelvis, and then the WHR was calculated as a measure of regional fat distribution.

All the data were analysed by SPSS 20.0. Data had been analysed for distribution and normality and appropriate statistics as be used. Test followed had been considered statistically significant if p < 0.05.

The following methods of statistical analysis have been used in this study. Continuous data were expressed as mean ± standard deviation and presented in tables and figures-

- 1) Paired Wilcoxon's signed rank.
- 2) Mann-Whitney independent samples test.
- 3) Test for normality- Shapiro-Wilk.

RESULTS

All the parameters were compared between 2 groups. There was as significant decreased in Hs-CRP, BMI, weight, WC and HC.

| Sl. No. | Hs-CRP (mg/dL) Pre | Post | Hs-CRP Chn |
|----------------------------|--------------------|-------------|--------------|
| Intervention Group (n= 15) | 0.53 ± 0.66 | 0.47 ± 0.5 | 0.07 ± 0.27 |
| Control Group (n= 15) | 0.32 ± 0.28 | 0.33 ± 0.33 | -0.02 ± 0.07 |

Table 1. Represents Mean Scores of both the Groups on the High Sensitivity C-Reactive Protein (Hs-CRP). Values are Mean ± Standard Deviation

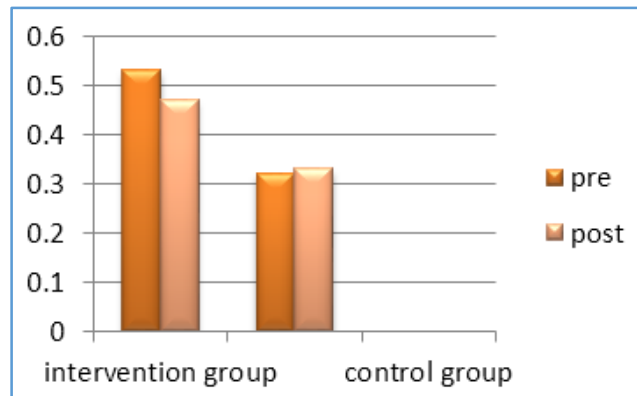
Hs-CRP= High sensitivity C-reactive protein; Hs-CRP Chn is the difference between pre and post mean value ± standard deviation.

Hs-CRP P= 0.426 as per paired Wilcoxon's signed rank within intervention group.

P= 0.378 as per paired Wilcoxon's signed rank within control group.

Hs-CRP P= 0.267 as per Mann-Whitney Independent sample test between groups.

Hs-CRP is not significant in this study. In intervention group, the difference between pre and post mean values ± standard deviation shows that there is decrease in Hs-CRP level in this study, but Hs-CRP level is increased in control group.



Graph 1. Comparing Hs-CRP in Intervention and Control Groups

This graph shows that Hs-CRP is decreased in intervention group, but in control Hs-CRP is increased.

| Sl. No. | BMI Pre | Post | BMI Chn | Weight (kg) Pre | Post | Weight Chn |
|--------------------|-------------|-------------|-----------|-----------------|-------------|------------|
| Intervention Group | 29.5 ± 3.2 | 27.5 ± 3.1 | 2.0 ± 0.4 | 72.5 ± 9.4 | 67.6 ± 8.8 | 4.92 ± 1.0 |
| Control Group | 28.2 ± 3.09 | 26.8 ± 3.17 | 1.4 ± 3.2 | 67.9 ± 8.86 | 64.6 ± 9.03 | 3.3 ± 0.7 |

Table 2. Represents Mean Scores of both the Groups on Anthropometric Measurements like BMI and Weight. Values are Mean ± Standard Deviation

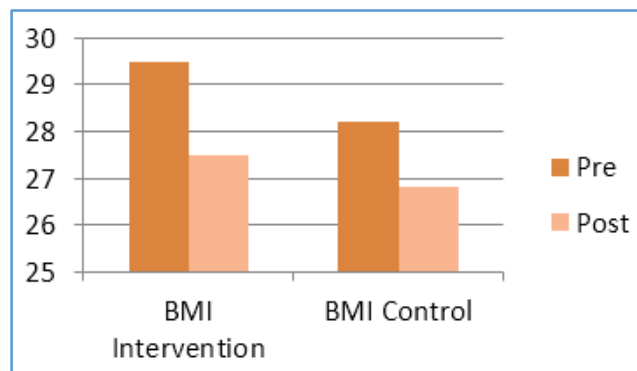
BMI= Body Mass Index.

For BMI P= 0.001, for Weight P= 0.001 as per paired Wilcoxon's signed rank within intervention group.

For BMI P= 0.001 for Weight P= 0.001 as per paired Wilcoxon's signed rank within control group.

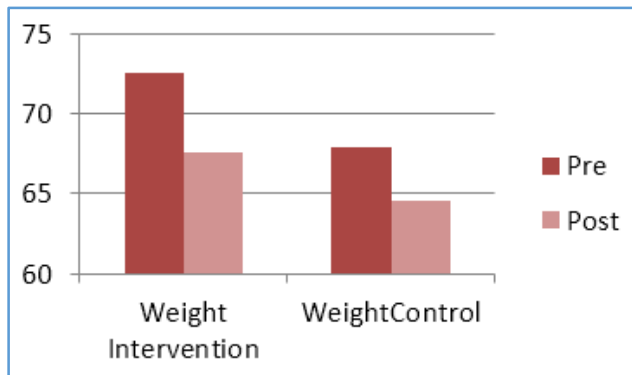
BMI P= 0.0001, Weight P= 0.0001 as per Mann-Whitney Independent sample test between groups.

BMI and Weight are highly significant in this study. BMI and Weight mean value ± standard deviation shows that there is decrease in both groups.



Graph 2. Comparing BMI in Intervention and Control Groups

This graph shows that BMI is decreased in both groups after giving intervention.



Graph 3. Comparing Weight in Intervention and Control Groups

This graph shows that BMI is decreased in both groups after giving intervention.

| Sl. No. | WC (cm) Pre | Post | WC Chn | HC (cm) Pre | Post | HC Chn |
|--------------------|--------------|-------------|--------------|--------------|-------------|-------------|
| Intervention Group | 92.2 ± 14.5 | 80.9 ± 13.2 | 11.27 ± 3.37 | 105.8 ± 8.39 | 95.2 ± 8.3 | 3.53 ± 25.0 |
| Control Group | 87.5 ± 13.04 | 79.4 ± 12.4 | 8.03 ± 2.43 | 108.3 ± 8.36 | 99.1 ± 8.16 | 2.0 ± 29.4 |

Table 3. Represents Mean Scores of both the Groups on the WC and HC. Values are Mean ± Standard Deviation

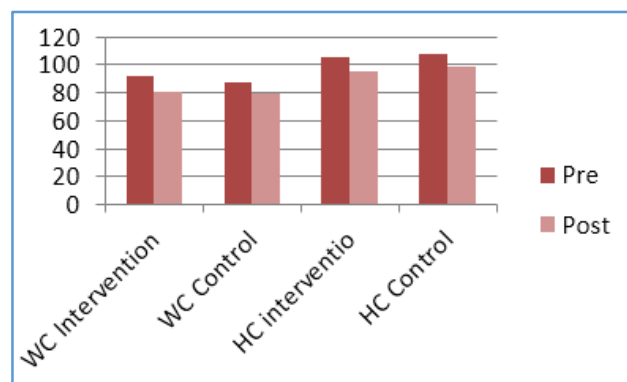
WC= Waist Circumference, HC= Hip Circumference.

For WC P= 0.001, for HC P= 0.001, for as per paired Wilcoxon’s signed rank within intervention group.

For WC P= 0.001, for HC P= 0.001, for as per paired Wilcoxon’s signed rank within control group.

WC P= 0.004, HC P= 0.004 as per Mann-Whitney Independent sample test between groups.

WC is highly significant in this study, but WC and HC mean value ± standard deviation shows that there is decrease in both groups.



Graph 4. Comparing WC and HC in Intervention and Control Groups

This graph shows that WC and HC is decreased in both groups after giving intervention.

| Sl. No. | WH Ratio Pre | Post | WH Ratio Chn |
|----------------------------|--------------|-------------|--------------|
| Intervention Group (n= 15) | 0.86 ± 0.1 | 0.84 ± 0.1 | 0.02 ± 0.04 |
| Control Group (n= 15) | 0.8 ± 0.08 | 0.79 ± 0.09 | 0.01 ± 0.29 |

Table 4. Represents Mean Scores of both the Groups on the WH Ratio. Values are Mean ± Standard Deviation

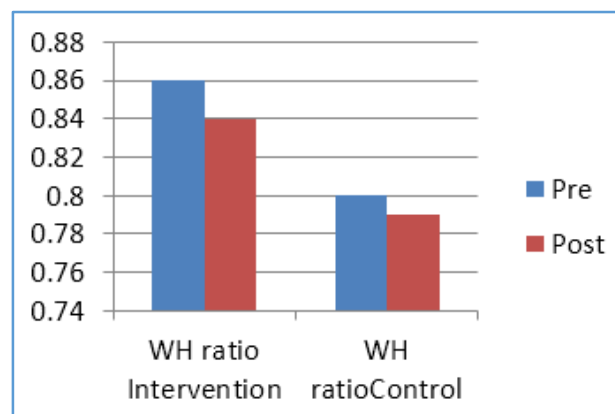
WHR= Waist-to-hip circumference ratio.

For WHR P= 0.123 as per paired Wilcoxon’s signed rank within intervention group,

For WHR P= 0.096 as per paired Wilcoxon’s signed rank within control group.

WHR P= 0.389 as per Mann-Whitney independent sample test between groups.

WHR mean value ± standard deviation shows that there is decrease in both groups.



Graph 5. Comparing WH Ratio in Intervention and Control Groups

This graph show that WHR is slightly decreased in both groups after giving intervention.

DISCUSSION

In this study, Hs-CRP level is reducing slightly. The present study is done in less subjects for shorter duration, so this study shows that Hs-CRP is not significant, but pre and post mean value ± standard deviation of Hs-CRP difference value of 0.0695 ± 0.27327 is decreased and this value is nearing to significant value, Hs-CRP results are compared with A. Salminen 2008 and Jin Hee Kim 2011 who suggested that terpenoids are suppressing the process of inflammation.^{26,27} In the present study inflammation marker result is same as A. Salminen and Jin Hee Kim study results on inflammation. Weight is significantly decreased and weight results are compared with Koji Nagao 2010, who had suggested that citrus auraptene given it for 4 weeks had decreased in TG levels and weight. The present study results of weight is same as Kiji Nagao results. BMI, WC and HC are significantly decreased in this study and WHR is not significant.

Limitation

In this study the sample size is low and it is taken as per our convenience not estimated scientifically.

CONCLUSION

The present study concluded that 7 days of lemon juice with lemon seeds extract, with calorie restriction has significantly decreased Hs-CRP, BMI, weight, waist circumference, hip circumference and WHR in overweight or obese subjects.

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