Effect of Psyllium Husk Based Dietetic Cookies on Hematological Parameters of Normal and Hypercholesterolemic Subjects

1Tahira Batool Qaisrani, 2Masood Sadiq Butt, 3Muther Mansoor Qaisrani, 4Ahmed H. El. Ghorab, 1Amjad Bashir, 1Tanveer Ahmed

1Assistant prof of Food Technology, Department of Agri Engg and Technology, Ghazi University, Pakistan
2Prof/Dean, National Institute of Food Science and Technology, Faculty of Food, Nutrition & Home Sciences
3Assistant Prof, Plant Biotechnology, Ghulam Fareed University of Technology, Rahim Yar Khan, Pakistan
4Prof, Faculty of science, Jouf University; Kingdom of Saudi Arabia
1Lecturer, Department of Plant Protection, Ghazi University, Pakistan
1Assistant Prof, Department of Horticultural Sciences, Ghazi University, Pakistan
*Corresponding Author: tqaisrani@gudgk.edu.pk

Abstract

Dietary fiber plays key role in the normal physiology of human. Psyllium husk is gaining attention as functional diet ingredient against hypercholesterolemia and its allied discrepancies. Cookies prepared with psyllium husk supplementation were administered to normal and hypercholesterolemic human subjects in two trials i.e., Trial-I and Trial-II. The objective was to efficacy and safety of psyllium husk as diet supplement in human. Results for hematological tests depicted safe range in allied parameters i.e., platelets count, ESR, red blood cells and white blood cells indices. It is interesting to mention that the ESR of hypercholestrolemic subjects was momentously affected by using therapeutic diet (T4: supplemented with 20% psyllium husk) as it was reduced to 5.61 and 6.98% in Trial-I & II, respectively. From the current explorations it is concluded that psyllium husk based cookies have potential to be used as a functional ingredient against the menace of hypercholesterolemia.

Keywords: Psyllium husk, Red Blood Indices, White Blood Indices, ESR, Hypercholesterolemia, Functional Foods

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INTRODUCTION

Changes in normal values of blood chemistry due to variation in physiological functioning of the body may lead to serious complications. Life style-related disorders like hypercholesterolemia, diabetes mellitus and hypertension problems may cause adverse changes in hematological parameters. Although the hypercholesterolemia is major risk factor for peripheral vascular diseases and coronary artery affecting large arterial vessels on the subject of atherosclerosis leading to ischemic heart disease [Ross, 1999]. Still it is leading factor to microvascular dysfunction creating reperfusion, inflated tissue injury and other stimuli like endotoxemia. The problems appear because of accumulation of the progressing and adhered platelets and leukocytes in the vessels [Granger, 1999; Stokes et al., 2002]. hypercholesterolemia may cause disturbance in hematological characters like platelets, erythrocyte sedimentation rate, lymphocytes, hemoglobin, hematocrit, total red blood cell count (TRBC), and neutrophils.

In USA, the National Cholesterol Education Program (NCEP) set guidelines for LDL<100 mg/dL and HDL not <40 mg/dL for prevention of stroke risk [Grundy, 2004]. The foremost reason is elevated levels of cholesterol and LDL whereas, HDL in blood is decreased than the recommended level. Plaques of cholesterol are formed and shrinkage of arteries may occur causing hindrance in normal blood flow thus ultimately leads to atherosclerosis [Gijsen et al., 2008]. Platelets adhesion in the micro vessels and lipid deposition (being the principal factor) are the possible mechanism behind. The atherosclerosis is the considerable threatening factor subsequent to CVDs and strokes. Atherosclerotic coronary artery disease has been at the front line in causing disability and death over the last couple of decades.

The strategies being implemented to cope with this malady are physical exercise, healthy diet and certainly the pharmaceuticals. Diet based therapies including food diversification, dietary supplementation and functional as well as nutraceuticals foods are helpful against the menace [Butt et al., 2009]. Dietary modification is an important tool as it is considered the frontline therapy. The vital features of the recommended changes in diet include restricting intake of total fat, saturated fatty acids, cholesterol and high glycemic foods. It has been observed that low glycemic foods are associated with regulation of HDL-cholesterol levels and reduce the incidence of hypercholesterolemia and cardiovascular diseases [Romero et al., 1998].

Dietary fiber is one of the valuable dietary interventions even to those who do not respond adequately to a low fat or low cholesterol diet [Anderson et al., 2000]. It also indicates that such products are associated with decreased risk of coronary disease [FDA, 1998]. Physiologically important psyllium husk fiber is consisting of a highly branched, arabinoxylan (AX) as its active fraction.

The use of soluble fiber in different therapies is significantly effective as a dietary intervention alone and in combination with medicines. The level of plasma triacylglycerol and HTR (High density lipoprotein cholesterol/total cholesterol) in diets containing cellulose, psyllium husk and psyllium husk along with Hydroxycitrate (HCA) were significantly lower than in the fiber free diets when studied by Kang et al. [2007]. They further analyzed that dietary supplementation with psyllium increased the total short chain fatty acid concentration when compared with cellulose supplemented diet. While, using the HCA with psyllium promoted the use of this mixture in making nutraceutical foods due to their fortified physiological activities.

The main objectives of present project include the endorsement of formulating psyllium fiber supplemented cookies for the vulnerable segment for managing health
disorders like hypercholesterolemia and allied discrepancies mainly related to hematological aspects.

**MATERIALS AND METHODS**

The study was carried out in two steps: 1. Trial I & Trial II. The efficacy trials were conducted in D. G. Khan Distt. after selecting normal and hypercholesterolemic volunteers. Psyllium husk was procured from Qarshi Industries (Pvt) Ltd. Pakistan. For product development commercial straight grade flour (CSGF) and other consumables/chemicals were acquired from the local market. The psyllium husk containing cookies were prepared in National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

**Efficacy studies**

The research was carried out in District Dera Ghazi Khan, Punjab, Pakistan for two consecutive years. For efficacy purpose, the volunteers were communicated regarding aims and perspectives of the study for consumer’s health viewpoint. They were also allowed to ask if they had any query about the product use. The written consent was taken from each volunteer to participate in the project till completion. Selection of subjects was done randomly on the basis of their anthropometric information, vital sign records and the baseline values of serum lipid profile and whole blood assay for study in normal and in hypercholesterolemic subjects. Selection of volunteers was made considering that there should be no significant differences in the base line values among normal individuals. The similar pattern was applied for the selection of hypercholesterolemic subjects. Diet schedule of each volunteer was also recorded to observe their eating behavior. The work plan for efficacy study is presented in Table 1.

Initially twenty normal subjects were selected on the criteria mentioned earlier. They were further divided in two groups of ten each. One group was provided with control cookies while other group consumed psyllium husk based cookies for two months study period. All the subjects were clinically examined on regular basis to appraise any difficulty during this period. In order to find out the effect of fiber supplementation the blood samples of subjects were drawn during the entire study. The study was repeated in next year for validity of results.

Similarly, hypercholesterolemic subjects were treated in same pattern as illustrated in normal subjects to evaluate the effect of dietetic cookies on the selected traits of blood indices. The trial for hypercholesterolemic subject was also repeated in next year.

**Blood sampling and determination**

Before the initiation of efficacy study, blood sample of each volunteer was collected for determination of base line values. Likewise, blood samples were also drawn on monthly basis up to two months to find out the effect of fiber supplementation.

**Blood assay**

Blood was examined for red blood cells indices, white blood cells indices, platelets count and erythrocytes sedimentation rate. Red blood cells indices included total red blood cells (TRBCs), hemoglobin (hb), hematocrit (Hct) and mean corpuscular volume (MCV) were determined by following the method of Al-Haj et al. [2011]. White blood cells indices comprised of white blood cells count (WBCs), lymphocytes, monocytes and basophils were determined following the protocol of Al-Haj et al. [2011]. Platelets count was carried out by the method of Thompson and Harker [1983] while erythrocyte sedimentation rate (ESR) was estimated by the procedure described by Widmann [1983].
Statistical analysis

The data obtained was subjected to statistical analysis using Cohort version 6.1 [Costat-2003]. Level of significance was estimated by using the analysis of variance technique (ANOVA) using two factor factorial CRD. Further, Tukey test was applied for comparing means [Steel et al., 1997].

Efficacy studies

Efficacy trials were carried out to explore the therapeutic potential of psyllium husk against hematological aspects of normal and hypercholesterolemic human subjects. The deviation in the results in human subject might be due to not always watching as in case of animal modeling where close supervision is done. The other reason might be intake of other diets.

Table 1: Efficacy study plan

<table>
<thead>
<tr>
<th></th>
<th>(Normal Subjects)</th>
<th>(Hypercholesterolemic Subjects)</th>
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<tbody>
<tr>
<td>Groups</td>
<td>G₁</td>
<td>G₁</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Cookies</td>
<td>D₁</td>
<td>D₁</td>
</tr>
<tr>
<td></td>
<td>D₂</td>
<td>D₂</td>
</tr>
</tbody>
</table>

D₁ = control  
D₂ = dietetic cookies

The planned modules were conducted in two consecutive years involving normal and hypercholesterolemic subjects by providing each subject with five cookies twice a day. One best treatment of cookies (T₄) was selected on the basis of physicochemical profile, dietary fiber content and sensory response along with control (T₀) for efficacy purpose. In each study, two groups of volunteers were formed with provision of control and dietetic cookies.

The individuals were tested for baseline values initially followed by sera analysis at 30 and 60 days to evaluate the potential of psyllium husk based cookies on selected parameters.

RESULTS AND DISCUSSION

Role of psyllium husk in altering the hematological traits was perceived. However, results of the investigated parameters in all studies are interpreted collectively for better understanding of the readers.

1. Platelets Count

Platelets count was affected non-significantly as function of treatments and study duration in both normal and hypercholesterolemic volunteers during the consecutive years (Table 2 and 3).

Means for platelets count in normal subjects (Trial-I) at initiation were 323.70±16.27 to 321.50±20.92K/μL as compared to 332.10±11.47 to 335.50±11.53K/μL at the termination of study in T₀ and T₄ groups, respectively. Similar trend was also observed in Trial-II for normal subjects. In hypercholesterolemic volunteers, values for platelets count ranged from 310.10±15.59 to 322.60±11.14K/μL and 319.50±14.21 to 328.90±11.31K/μL at 0 and 60 days in Trial I in the respective treatments. Likewise pattern was observed in Trial-II for this trait.

Collectively, the means for platelets count in normal individuals were 327.50±2.46 to 329.27±4.08K/μL and 322.71±3.35 to 324.34±4.59K/μL whereas in hypercholesterolemic subjects 318.37±4.13 to 328.00±4.67K/μL and 319.46±4.44 to 328.78±4.86K/μL for T₀ and T₄ groups, respectively during the entire study.
2. **Erythrocytes sedimentation rate (ESR)**

Non-significant variations were observed due to treatments and time intervals on erythrocytes sedimentation rate (ESR) in normal subjects whereas significant differences were found in hypercholesterolemic subjects (Table 2 and 3).

The mean values for ESR in normal subjects (Trial I) were 12.90±0.32 to 13.60±0.48mm/hr compared to 14.80±0.84 to 14.40±0.42mm/hr at 0 and 60 days in T0 and T4 groups, respectively. Similar pattern was observed in Trial II for this trait. However, in hypercholesterolemics (Trial-I), ESR ranged from 15.20±0.38, 15.31±0.65 and 15.36±0.54mm/hr whereas, 15.14±0.86, 14.71±0.62 and 14.29±0.41mm/hr at 0, 30 and 60 days in T0 and T4 groups, respectively. After consumption of psyllium husk based cookies (T4) significantly reduced ESR in hypercholesterolemic subjects (Trial-II) as 13.18±0.38mm/hr at 60 days was recorded compared to base line value of 14.17±0.80mm/hr.

![Figure 1. Percent change in ESR with psyllium based cookies](image)

Overall means regarding ESR presented in normal subjects varied from 13.20±0.21 to 14.33±0.29mm/hr and 13.02±0.23 to 14.08±0.30mm/hr whilst, in hypercholesterolemic subjects values for this parameter were 15.29±0.05 to 14.71±0.25mm/hr and 14.81±0.26 to 13.62±0.29mm/hr in T0 and T4 groups, respectively during the entire study. Percent reduction for ESR due to T4 was observed from 2.71 to 2.88% in normal subjects while 5.61 to 6.98% in hypercholesterolemic individuals after 60 days study period as compared to baseline values for Trial I & II, respectively (Fig. 1).

**White blood cells indices**

1. **White blood cells count (WBCs)**

Statistically, total WBCs count explicated non-significant differences due to treatments and study period in normal and hypercholesterolemic subjects in Trial I and Trial II (Table 2 and 3).
Means for total WBCs count in normal individuals (Trial-I) at the start were 6.14±0.39 to 6.15±0.34 K/μL that differed non-significantly to 5.90±0.28 to 6.36±0.49 K/μL in T₀ and T₄ groups during two months trials, respectively. In hypercholesterolemic subjects (Trial-I), WBCs count in T₀ and T₄ groups at 1st day were 7.33±0.47 and 7.23±0.40 K/μL that decreased non-significantly as 7.73±0.37 to 6.87±0.53 K/μL in respective treatments after 60 days. Similar trend was observed in both normal and hypercholesterolemic subjects in Trial-II.

2. Neutrophils

Non-momentous differences due to treatments and study period on neutrophils count in normal and hypercholesterolemic subjects (Table 2 and 3) were noticed during the study period.

Means for neutrophils at beginning of study were 59.30±1.21 and 61.20±1.93% in normal subjects (Trial-I) relying on T₀ and T₄ treatments, respectively. After two months, the values were 62.70±1.69 and 65.70±1.68% in respective groups. In hypercholesterolemic individuals (Trial-I) neutrophils count initially noted were 67.30±1.38 and 63.80±1.14% compared to 69.00±1.07 and 65.80±1.69% at 60 days in T₀ and T₄ groups, respectively. Likewise trend for this trait in normal and hypercholesterolemic subjects was observed in the up-coming year i.e. Trial II. Collectively, the means for neutrophils in normal subjects ranged from 61.07±0.98 to 63.57±1.30% and 61.43±0.86 to 63.94±1.22% whereas values were 67.30±1.38 to 63.80±1.14% and 66.86±1.37 to 63.30±1.79% in hypercholesterolemic subjects for T₀ and T₄ treated groups, respectively during entire study.

3. Lymphocytes

Statistical data explicated non-momentous variations due to treatments and study duration on lymphocytes in both normal and hypercholesterolemic subjects (Table 2 and 3).

Means for lymphocytes indicated non-significant variations in normal subjects (Trial-I) as 31.90±1.33 to 33.50±1.55% and 32.20±1.81 to 33.90±1.22% at 0 and 60 days in groups relying on T₀ and T₄ treatments, respectively. Similarly, in hypercholesterolemic individuals (Trial-I) non-significant values were observed ranging from 31.00±1.24 to 32.20±1.84% and 32.50±1.84 to 33.80±1.21% at 0 and 60 days in T₀ and T₄ groups, respectively. Similar findings for lymphocytes were observed in both normal and hypercholesterolemic subjects during the Trial-II. Overall means regarding lymphocytes in normal subjects ranged from 32.73±0.46 to 33.13±0.50% and 32.85±0.52 to 33.27±0.53% whereas, in hypercholesterolemic subjects values for this parameter were 32.07±0.58 to 33.83±0.78% and 31.81±0.59 to 33.71±0.84% during the selected years.

4. Monocytes

Data exhibited non-significant differences on monocytes percentage as function of treatments and study intervals in normal and hypercholesterolemic individuals during two consecutive years (Table 2 and 3).

Monocytes in normal individuals, monocytes concentration (Trial-I) at initiation of the study were 4.90±0.29 and 5.50±0.29% that altered to 5.10±0.33 and 5.60±0.31% at termination of study in T₀ and T₄ groups, respectively. The monocytes in hypercholesterolemic individuals (Trial-I) at beginning of study were 4.50±0.26 and 5.30±0.28% that changed to 4.80±0.31 and 5.70±0.31% at 60 days in T₀ and T₄ groups, respectively. Similar trend was observed in both normal and hypercholesterolemic subjects in Trial-II. Collectively, the means for monocytes varied from 5.20±0.21 to 5.43±0.12% and 5.22±0.21 to 5.46±0.11% in normal subjects while 4.83±0.20 to 5.77±0.29% and 4.81±0.20 to
5.74±0.29% in hypercholesterolemic individuals in T₀ and T₄ groups, respectively during the entire study period.

**Red blood cells indices**

1. **Red blood cells (RBCs) count**

   Statistical analysis for red blood cells count indicating non-significant variations as function of treatments and time duration regardless of subjects during the study (Table 4 and 5).

   Means for RBC in normal subjects (Trial I & II) were 5.33±0.19 to 5.53±0.23M/µL and 5.41±0.16 to 5.13±0.18 M/µL at 0 and 60 days in T₀ and T₄ groups, respectively. Similar trend for this parameter was observed in normal subjects in Trial II. In hypercholesterolemic (Trial-I), values for RBC were 5.52±0.19 to 6.90±0.29M/µL and 5.78±0.17 to 6.05±0.21 M/µL at 0 and 60 days in respective treatments. Likewise pattern for this trait was observed in Trial-II. Overall, means for RBCs count were 5.23±0.21 to 5.30±0.09M/µL and 5.25±0.07 to 5.28±0.13M/µL whereas, 5.86±0.13 to 5.87±0.09M/µL and 5.56±1.30 to 5.99±0.08M/µL in T₀ and T₄ treatments for both normal and hypercholesterolemic subjects, respectively during Trial I & II.

2. **Hemoglobin level (Hb)**

   The hemoglobin level depicted that treatments and duration affected the Hb level non-significantly in normal and hypercholesterolemic subjects during the study (Table 4 and 5).

   Data for hemoglobin concentration explicated that in normal subjects (Trial-I) the values were 13.78±0.77 to 13.66±0.8g/dL and 14.19±0.48 to 13.87±0.51g/dL at 0 and 60 days in T₀ and T₄ groups, respectively. Similarly, in hypercholesterolemic volunteers (Trial-I) the means for this trait were 14.08±0.79 to 14.98±0.38 g/dL and 15.60±0.67 to 14.72±0.30g/dL at initiation and termination of study in respective treatments. Likewise pattern was observed in both normal and hypercholesterolemic subjects (Trial-II). Overall means regarding hemoglobin varied from 13.77±0.06 to 14.27±0.26g/dL and 13.89±0.10 to 14.27±0.26g/dL in normal volunteers whereas, in hypercholesterolemic subjects values were 14.68±0.30 to 15.16±0.26g/dL and 14.70±0.16 to 14.62±0.25g/dL in T₀ and T₄ groups, respectively during two consecutive years.

3. **Hematocrit**

   Statistical results for hematocrit revealed non-significant differences due to treatments and study intervals in normal and hypercholesterolemic individuals in Trial-I & II (Table 4 and 5).

   In normal subjects (Trial-I), means for hematocrit at start of the study were 45.90±1.12 and 45.10±1.56% as compared to 47.50±1.26 and 44.20±1.53% at 0 and 60 days in T₀ and T₄ groups, respectively. Similar behavior regarding hematocrit concentrations was estimated in Trial II. In hypercholesterolemic subjects the means recorded for T₀ and T₄ groups at the initiation were 47.82±1.20 and 47.70±1.65% while at completion of trial values as 59.87±1.59 and 52.38±1.75% in T₀ and T₄ groups, respectively during Trial I. Overall means regarding hematocrit in normal subjects varied from 46.47±0.52 to 45.13±0.55% and 47.63±0.43 to 44.77±0.55% whereas, in hypercholesterolemic subjects values were 52.42±1.76 to 50.06±1.35% and 51.33±0.38 to 49.10±0.26% in T₀ and T₄ groups, respectively during the entire study period.

4. **Mean corpuscular volume (MCV)**

   Statistical analysis indicated non-significant effect of treatments and duration on mean corpuscular volume (MCV) in both groups during the selected period (Table 4 and 5).
Means for MCV in normal subjects (Trial-I) at 0 and 60 days were 86.12±2.94 to 85.90±5.46fL and 83.36±3.29 to 86.16±5.92fL whereas in hypercholesteroleemics (Trial-I) values for this trait were 88.64±5.95 to 89.96±1.77fL and 88.84±3.44 to 83.08±4.92fL in T₀ and T₄ groups, respectively. Similar trend for MCV was observed during Trial-II for respective groups. Collectively, the means for MCV varied from 89.15±3.14 to 85.18±0.91fL and 90.68±1.11 to 84.80±1.61fL in normal subjects whilst, the values for this parameters ranged from 89.47±0.42 to 86.16±1.68fL and 91.54±0.28 to 80.65±0.45fL in hypercholesterolemic subjects in T₀ and T₄ groups, respectively in Trial I & II.

Discussion

In this context, Tailor and Granger [2004] worked on wild type mice and reported increased platelets adhesion during hypercholesterolemia owing to hematological disturbances leading to athrogenesis. Considering hypercholesterolemia, the principal factor behind is atherosclerosis. Liu et al. [2007] explicated that microvesicles formation may increase up to three folds in hypercholesterolic individuals due to excessive cholesterol supplementation of monocytes. Similarly, Choi and Pai [2004] worked on the hematology of normal and hyperlipidemic subjects and reported increased ESR in hypercholesterolemic adults compared to normal subjects while reporting non-momentous differences in mean corpuscular volume between respective groups.

The results regarding hematological aspects are showing some contradictions with the finding of Jenkins et al. [2007] as they indicated reduction in neutrophils, hematocrit, TRBC and hemoglobin after taking the cholesterol lowering diet although the platelets count was not affected. Considering the safety aspects of psyllium husk, Prasad [2005] determined that persistent use of dietary fiber from flax seed (lignan) did not disturb the blood system as it showed no adverse effect on white blood cells indices, red blood cells indices and platelet count in normal and hypercholesterolemic individuals.

Similarly, Carabin et al. [2009] reported non-significant differences in biochemical and hematological parameters in normal subjects after consuming the product “Polyglycoplex” as source of fiber. The effect of psyllium fiber added diet on the C-reactive protein and WBC count of obese elderly people was studied by King et al., [2008]. The statistical results revealed non significant differences between the groups taking 7 or 14g/d psyllium fiber for 3 months or when compared with group without psyllium fiber supplementation. The antioxidant and dietary fiber content from Mediterranean diet exhibit inverse relationship with WBC count and to some extent accounted for the involvement with PLTs count [Bonaccio et al., 2014].

CONCLUSION

Psyllium husk manages ESR in normal subjects while improves ESR status in hypercholesterolemic volunteers proving its functional worth against the physiological threats. Psyllium husk based diet proved safe due to normal hematological values. In the nutshell, the therapeutic food containing psyllium husk is effective to control dyslipidemia and allied discrepancies including hematological parameters. It is inferred through the discussion that psyllium husk enriched foods may be introduced in diet based therapy to combat lifestyle-related disorders.

REFERENCES


Table 2. Mean squares for effect of dietetic cookies on Platelet count, ESR and WBCs indices of normal subjects

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Platelet count</th>
<th>ESR</th>
<th>WBC</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<tr>
<td></td>
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<td>T-I</td>
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<td>T-I</td>
<td>T-II</td>
<td>T-I</td>
<td>T-II</td>
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<tr>
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<td>912.29NS</td>
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<td>A × B</td>
<td>2</td>
<td>60.50NS</td>
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Table 3. Mean squares for effect of dietetic cookies on Platelet count, ESR and WBCs indices of hypercholesterolemic subjects

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Platelet count</th>
<th>ESR</th>
<th>WBC</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<td>T-II</td>
<td>T-I</td>
<td>T-II</td>
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<td>46.18NS</td>
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<td>19.15*</td>
<td>0.86NS</td>
<td>0.81NS</td>
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<td>Duration (B)</td>
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<td>92.49NS</td>
<td>2.66*</td>
<td>1.60*</td>
<td>0.30NS</td>
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<td>A × B</td>
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<td>575.68NS</td>
<td>947.97NS</td>
<td>12.69*</td>
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83
Table 4. Mean squares for effect of dietetic cookies on RBCs indices of normal subjects

<table>
<thead>
<tr>
<th>SOV</th>
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<th>RBCs count</th>
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<th>Hematocrit</th>
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<td>T-I</td>
<td>T-II</td>
<td>T-I</td>
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<tr>
<td>A × B</td>
<td>2</td>
<td>1.102&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.044&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.591&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.940&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>54</td>
<td>0.0405</td>
<td>0.039</td>
<td>0.435</td>
<td>0.423</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>59</td>
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<td></td>
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</table>

Table 5. Mean squares for effect of dietetic cookies on RBCs indices of hypercholesterolemic subjects

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>RBCs count</th>
<th>Hemoglobin</th>
<th>Hematocrit</th>
<th>MCV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T-I</td>
<td>T-II</td>
<td>T-I</td>
<td>T-II</td>
</tr>
<tr>
<td>Treatments (A)</td>
<td>1</td>
<td>0.002&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.010&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.47&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.095&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration (B)</td>
<td>2</td>
<td>5.69&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.028&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>A × B</td>
<td>2</td>
<td>2.96&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.262&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.26&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.36&lt;sup&gt;NS&lt;/sup&gt;</td>
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<tr>
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<td>0.07</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>Total</td>
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