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# BENEFICIAL EFFECTS OF MILK KEFIR IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

**Basant M Morsy[1], Ayman M Mahmoud[2*], Mohamed I Zanaty[1], Adel Abdel-Moneim[2] and Mohamed A Abo-Seif[3]**

[1]Biochemistry Division, Chemistry Department, [2]Physiology Division, Zoology Department, Faculty of Science, [3]Department of Internal Medicine, Faculty of Medicine, Beni-Suef University, Egypt.

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**Abstract:** The current study was designed to evaluate the beneficial therapeutic effects of milk kefir in Egyptian hepatitis C virus (HCV) patients. Thirty volunteer patients with proven HCV and fifteen age matched healthy subjects were included in this study. Exclusion criteria included patients on interferon therapy, infection with hepatitis B virus, drug-induced liver diseases, advanced cirrhosis, hepatocellular carcinoma or other malignancies, blood picture abnormalities and major severe illness. The included subjects were divided into three groups as follows: Group 1 served as healthy, Group 2 served as HCV control and Group 3 HCV patients received 250 ml freshly prepared milk kefir twice daily for one month. Liver function enzymes, albumin, total bilirubin, prothrombin time and concentration, international normalized ratio, lipid profile and viral load were all assessed at baseline and at the end of the study. Milk kefir exhibited marked therapeutic benefits in HCV patients through decreasing viral load, alleviating the altered liver function and improvement of lipid profile. The ameliorative effects of milk kefir in HCV patients may be attributed to its antioxidant, anti-inflammatory and immune-stimulatory effects.

**Key Words:** Hepatitis C Virus, Kefir, Viral Load.

## INTRODUCTION

Hepatitis C is a major global health burden with an estimated 160 million infected individuals worldwide. This long-term disease evolves slowly, often leading to chronicity and potentially to liver failure[1]. According to the World Health Organization (WHO) there are 130-170 million people infected with the hepatitis C virus, corresponding to 2-2.5% of the world's total population[2]. Egypt has the highest prevalence of hepatitis C virus worldwide (15%) and the highest prevalence of HCV-4 (67%) with a predominance of subtype 4a (55%)[2,3]. It has been reported that HCV infection often advances to chronic hepatitis due to the low viral clearance rate, leading to liver cirrhosis (LC) and subsequent development of hepatocellular carcinoma (HCC)[4,5].

The standard therapy, which is based on a combination of pegylated interferon alpha (IFN-α) and ribavirin[6], results in highly variable outcomes, is very expensive and has severe side effects that are difficult to endure for the patients[1,7]. Therefore, the establishment of a new treatment modality without serious adverse effects is desirable[8].

Kefir is an Old World food that has been attributed with exceptional health promoting and curative properties since the beginning of recorded history. It is a tangy, slightly fizzy, fermented milk beverage that looks a little like yogurt. It is rich in protein, calcium, vitamin $B_{12}$, niacin, and folic acid[9]. Kefir drink is a popular natural probiotic beverage in the Middle East. It is the fermentation product of a variety of probiotic microorganisms live microorganisms added to the diet in sufficient quantities to improve the health condition- present in the kefir grain of the milk[10].

Several studies suggest beneficial health effects of kefir grains, including antitumor[11], antimicrobial, antioxidant[12], immunomodulatory[13], anti-inflammatory[14] and gastrointestinal regulatory activities[15]. Therefore, the current study was designed to evaluate the beneficial effects of milk kefir in chronic hepatitis C patients.

## MATERIALS AND METHODS

### *Preparation of milk kefir*

Milk kefir was prepared by adding kefir grains (2-10%) to milk that have been pasteurized and cooled to 20-25°C. After 24 h of fermentation period at 25°C, the grains were separated from by filtration through a plastic sieve and washed prior to the next culture incubation.

### *Patients*

Thirty volunteer patients with proven chronic hepatitis C virus who were selected from admitted patients at the Hepatology Unit, Beni-Sueif University Hospital, with fifteen age-matched healthy controls, were included in the study.

***Corresponding Author:**
**Dr. Ayman M Mahmoud,**
Physiology Division, Zoology Department,
Faculty of Science, Beni-Suef University, Egypt.
E-mail: ayman.mahmoud@science.bsu.edu.eg

Inclusion criteria included all patients diagnosed with HCV and negative for HBV. Exclusion criteria included patients on IFN-α therapy; infection with HBV or hepatitis virus; drug-induced liver diseases; advanced cirrhosis; HCC or other malignancies; blood picture abnormalities such as anemia (hemoglobin concentration of 10 g/dl or less), leucopenia (white blood cells 1.500/µl or less) and thrombocytopenia (platelets count 80.000/µl or less); major severe illness such as renal failure, congestive heart failure, respiratory failure or autoimmune disease; or non-compliance to treatment. Informed consent was obtained from all patients, and the institutional ethical committee approved the study protocol, which conformed with the ethical guidelines of the 1975 Declaration of Helsinki.

***Treatment protocol***

Included patients and controls were classified into three groups each comprising fifteen subjects as follows; Group 1 served as healthy subjects; Group 2 (HCV) served as HCV control; Group 3 (HCV + Kefir) received 250 ml freshly prepared milk kefir twice daily. Patients were followed up weekly throughout the study period for assessing treatment adherence, tolerability, and incidence of adverse reactions.

***Clinical and laboratory assessment***

All eligible patients and controls were subjected to the following at enrollment and after 1 month therapy: (1) Full clinical assessment with an emphasis on hepato- and/or splenomegaly, jaundice, palmar erythema, flapping tremors, spider naevi, lower-limb edema, and ascites; (2) Laboratory investigation of liver functions including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed according to the method of Schumann and Klauke[16] using reagent kit purchased from Noble Diagnostic (Egypt); lactate dehydrogenase (LDH) was determined according to the method of Teitz[17] using reagent kit purchased from Human (Germany); alkaline phosphatase (ALP) was assayed according to the International Federation of Clinical Chemistry (IFCC)[18] using BioSystem (Spain) commercial kit, gamma glutamyl transferase (γGT) performed according to Persijn and van derSlik[19] using kits of Reactivos GPL (Spain); albumin was assayed according to the method of Webster[20] using kits of Diamond Diagnostic (Egypt); total bilirubin, was determined using reagent kit purchased from Diamond Diagnostic (Egypt), according to the method of Kaplan *et al.*,.[21] and prothrombin time (PT), prothrombin concentration (PC) and international normalized ratio (INR) according to the method of Wanger and Dati[22] using kits of Siemens Healthcare Diagnostic (USA).

Total cholesterol[23], triglycerides[24] and HDL-cholesterol[23] were assayed using reagent kit purchased from Spinreact (Spain). Serum LDL-cholesterol level was calculated from Friedewald[25] formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol). Serum vLDL-cholesterol concentration was calculated according to Nobert[26] formula (vLDL-cholesterol = triglycerides/5). Cardiovascular risk indices were calculated according to Ross[27] formula as follows: cardiovascular index 1 = total cholesterol/HDL-cholesterol and cardiovascular index 2 = LDL-cholesterol/HDL-cholesterol. Quantitative real time polymerase chain reaction (qPCR) for HCV using Roche Amplicor HCV monitor version 2.0 (Roche Diagnostics, Branchburg, NJ, with lower detection limit < 50 IU/ml).

***Statistical analysis***

Statistical analysis was performed using SPSS v.16. Results were articulated as mean ± SE and all statistical comparisons were made by means of one-way ANOVA test followed by Duncan's multiple range test post hoc analysis. A P value <0.05 was considered significant.

## RESULTS

The effect of milk kefir on viral load in control and treated patients was represented in Figure 1. After one month treatment with milk kefir, patients exhibited a significant decrease in viral load as compared to HCV group. The recorded data were 244.13 ± 36.72 and 390.33 ± 80.12 for kefir-treated and HCV control patients, respectively.

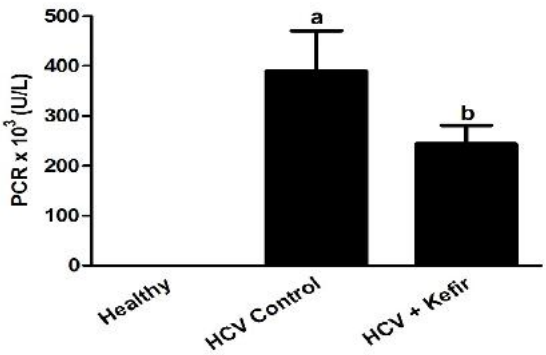


**Figure 1:** Viral load of control and treated patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.001.

The activities of serum liver enzymes AST, ALT, γGT, LDH and ALP were summarized in Table 1. HCV control patients exhibited significantly elevated serum activities of all assayed enzymes when compared to corresponding healthy subjects. Administration of milk kefir significantly (P<0.001) alleviated the elevated liver enzymes.

**Table 1:** Serum liver enzymes of healthy, HCV control and HCV treated with kefir.

| Parameter / Group | ALT (U/L) | AST (U/L) | LDH (U/L) | γGT (U/L) | ALP (U/L) |
|---|---|---|---|---|---|
| Healthy | 21.73 ± 1.67$^{c}$ | 23.33 ± 0.85$^{c}$ | 255.27 ± 9.60$^{c}$ | 16.13 ± 1.47$^{c}$ | 72.20 ± 2.94$^{b}$ |
| HCV control | 54.87 ± 4.80$^{a}$ | 49.40 ± 3.84$^{a}$ | 454.07 ± 25.91$^{a}$ | 59.53 ± 4.10$^{a}$ | 112.33 ± 9.01$^{a}$ |
| HCV + Kefir | 44.47 ± 2.29$^{b}$ | 40.60 ± 2.21$^{b}$ | 378.13 ± 12.23$^{b}$ | 42.07 ± 1.36$^{b}$ | 71.87 ± 2.93$^{b}$ |

Data are expressed as mean ± SE. Number of patients in each group is fifteen. Means which share the same superscript symbol(s) are not significantly different, P<0.001.

Conversely, serum albumin of HCV control group showed a significant decrease in comparison to healthy control group as showed in Table 2. On the other hand, serum total bilirubin exhibited an opposite behavioral pattern; it was significantly elevated in HCV control patients as compared to their corresponding healthy subjects (Table 2). Similarly, PT and INR were significantly elevated in HCV control group, while PC was significantly declined. Supplementation of milk kefir produced marked improvement of albumin, bilirubin, PT, PC and INR as depicted in Table 2.

**Table 2:** Albumin, total bilirubin, PT, PC and INR of healthy, HCV control and HCV treated with kefir.

| Parameter / Group | Albumin (g/dl) | Bilirubin (mg/dl) | PT (Sec) | PC (%) | INR |
|---|---|---|---|---|---|
| Healthy | 4.57 ± 0.09$^{a}$ | 0.21 ± 0.03$^{c}$ | 12.86 ± 0.06$^{c}$ | 95.68 ± 0.65$^{a}$ | 1.05 ± 0.01$^{c}$ |
| HCV control | 3.69 ± 0.09$^{c}$ | 0.83 ± 0.08$^{a}$ | 13.69 ± 0.19$^{a}$ | 88.61 ± 1.47$^{c}$ | 1.13 ± 0.02$^{a}$ |
| HCV + Kefir | 4.05 ± 0.04$^{b}$ | 0.66 ± 0.04$^{b}$ | 13.29 ± 0.10$^{b}$ | 91.77 ± 0.89$^{b}$ | 1.09 ± 0.01$^{b}$ |

Data are expressed as mean ± SE. Number of patients in each group is fifteen. Means which share the same superscript symbol(s) are not significantly different, P<0.001.

The data represented in Figures 2-6 showed that milk kefir supplementation for one month produced significant ameliorations of serum total cholesterol (P<0.01), triglycerides (P<0.01), HDL- (P<0.05), LDL- (P<0.05), and vLDL-cholesterol (P<0.01). Similarly, treatment of HCV patients with milk kefir significantly (P<0.05) decreased the cardiovascular risk indices of HCV treated patients compared to the HCV control ones (Figures 7 & 8).

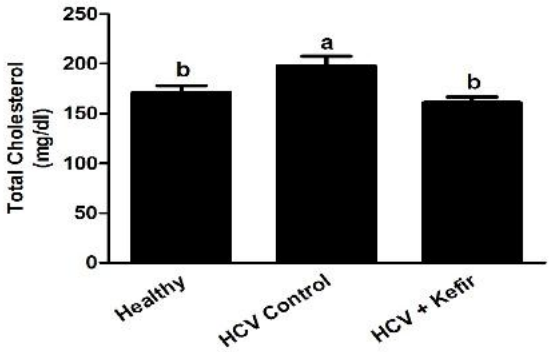


**Figure 2:** Serum total cholesterol of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.01.

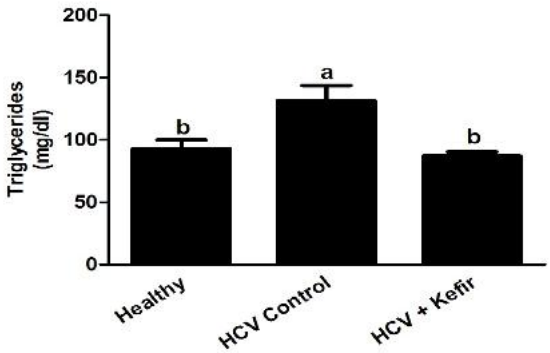


**Figure 3:** Serum triglycerides of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.01.

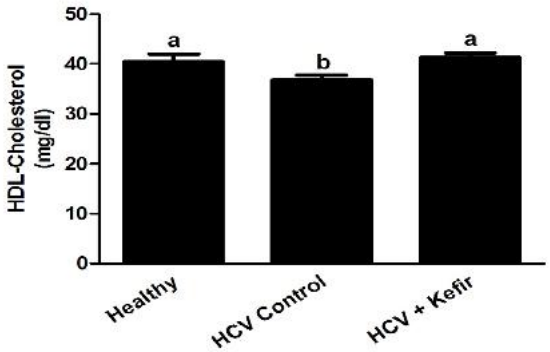


**Figure 4:** Serum HDL-Cholesterol of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.05.

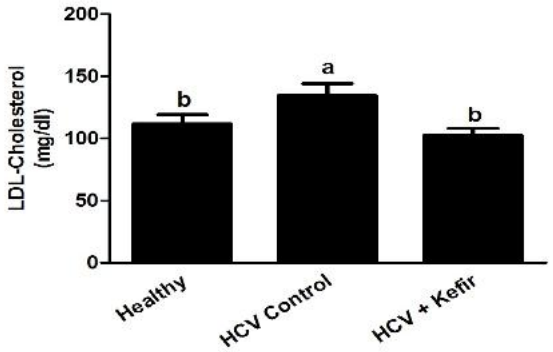


**Figure 5:** Serum LDL-Cholesterol of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.05.

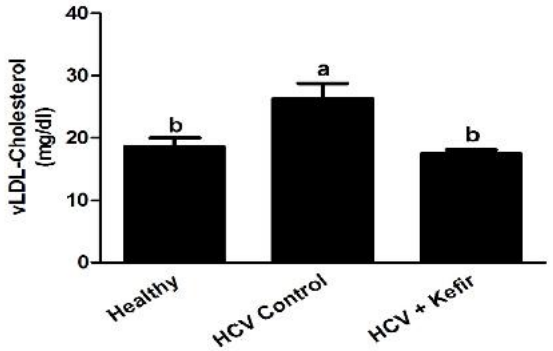


**Figure 6:** Serum vLDL-Cholesterol of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.01.

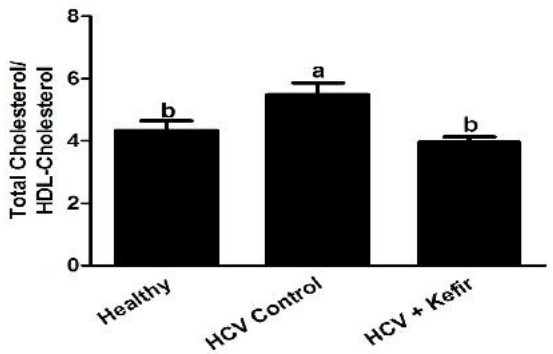


**Figure 7:** Cardiovascular risk index 1 of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.05.

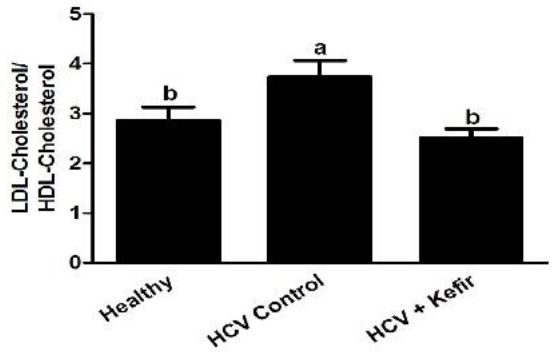


**Figure 8:** Cardiovascular risk index 2 of healthy, HCV control and Kefir-treated HCV patients. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, P<0.05.

## DISCUSSION

Hepatitis C is a major global health burden and Egypt has the highest prevalence of hepatitis C virus worldwide[28]. Given the high cost of the drugs, especially in low-income countries with a high prevalence of HCV, it is desirable to search for low cost and safe remedies for successful HCV treatment. In this concern, several studies performed on various liver diseases have confirmed the positive influence of probiotic strains on pathophysiological symptoms as reviewed by Imani Fooladi *et al.*,[29] They concluded that a balanced and healthy gut prevents a high percentage of harmful liver conditions. Therefore, the current study was designed to evaluate efficacy, safety, and tolerability of milk kefir in Egyptian patients with chronic HCV.

The role of liver enzymes in the assessment of chronic hepatitis C remains important due to the fact that the majority of clinical indexes estimating the degree of liver fibrosis are based in liver transaminases[30]. The present investigation showed a significant increase in serum ALT, AST, LDH, γGT and ALP activities of untreated HCV patient as compared to healthy control group. These enzymes are considered the most sensitive markers of liver injury as they are found in the cytoplasm of liver cells, thus damage of these cells lead to their rapid leakage into the blood circulation[31]. The significantly elevated liver enzymes could be attributed to viral-induced hepatocellular damage[28].

The current results showed that milk kefir supplementation potentially alleviated the serum marker enzymes of liver function; ALT, AST, LDH, ALP and γGT. In addition, supplementation of milk kefir to chronic hepatitis C patients markedly decreased viral load when compared to the corresponding control patients. The ameliorative effects of milk kefir may be

attributed to its antioxidant[12], anti-inflammatory[14] and immune-stimulatory effects[13]. Stimulation of the immune system might be one mechanism whereby probiotic bacteria may exert many of their beneficial effects[32]. In addition, stimulation of the immune system may be attributed to the action of exopolysaccharides found in kefir grains as stated by Murofushi *et al.*,[33]

Serum albumin and prothrombin time can be considered useful tools, alone or combined in clinical scores, for evaluating liver function[34]. Chronic HCV patients may suffer a decrease in serum albumin level[35], and improvement in hypoalbuminemia has been shown to improve prognosis[36] and quality of life[37]. In the current study, administration of milk kefir significantly increased serum albumin levels and decreased the elevated prothrombin time as well as serum bilirubin levels, indicating an improvement in clinical condition.

A study conducted by Alter and Mast[38] revealed that hyperlipidemia has been reported in patients with chronic HCV infection. In addition, HCV infected subjects were at a significantly higher risk of developing coronary artery disease, compared with HCV-uninfected subjects, even after adjustment for traditional risk factors for cardiovascular disease[39]. The current findings demonstrated that supplementation of milk kefir potentially alleviated the altered lipoprotein profile of HCV patients. Kefir supplementation increased HDL-cholesterol and markedly decreased the elevated serum triglycerides, total cholesterol and LDL and vLDL cholesterol levels. In consistent, the study of Liu *et al.*,[40] demonstrated an increase in HDL-cholesterol on using milk-fermented or sugar-fermented kefir preparations. In addition, supplementation of milk kefir markedly decreased the cardiovascular risk indices, indicating it's anti-hyperlipidemic and cardio protective efficacies.

## CONCLUSION

The current study demonstrated that kefir exhibited marked therapeutic benefits in HCV patients through decreasing viral load, ameliorating the altered liver function and improvement of lipid profile.

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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