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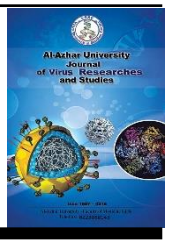

Effect of Fenugreek, Nigella Sativa and Green Tea Mixture on Liver Function, Oxidative–Antioxidant Balance and Vitamin D Metabolism in Patients with HCV-Related Liver Cirrhosis

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Abstract

Hepatitis C virus (HCV) infection is a major cause of morbidity and mortality that is responsible for hepatic inflammation and the formation of scar tissue (fibrosis) by release of free radicals and impaired vitamin D metabolism. We aimed to assess the effect of herbal therapy on serum vitamin D and free radicals and antioxidants in patients with HCV-related liver cirrhosis (LC). The study incorporated 48 patients with HCV-related LC and 22 healthy controls. Out of cirrhotics, 24 patients received one spoonful of herbal mixture composed of equal volumes of mixed powder of fenugreek, Nigella sativa and green tea two times daily for 2 months. Liver function tests, serum oxidative stress malonaldahyde (MDA), antioxidant superoxide dismutase (SOD), reduce glutathione (GSH), vitamin D (25OH-D3) and parathyroid hormone (PTH) were estimated. Significantly improved liver function, 25OH-D3 and antioxidants (SOD, GSH) and decreased oxidative stress indicator (MDA) levels in herbal treated cirrhotics than non-herbal treated patients but they still impaired compared to healthy controls. Herbal administration may attain significant improvement in liver function, oxidative–antioxidant balance and vitamin D metabolism in patients with HCV- related LC.

Keywords: Liver disease, Herbal Treatment-25OH-D3, PTH.

1. Introduction

Hepatitis C virus (HCV) infection is the major etiological agent of chronic hepatitis and liver disease worldwide. About 130-170 million people infected with the hepatitis C virus, corresponding to 22.5% of the world's total population [1] Egypt has the highest prevalence of HCV worldwide (15%) and the highest prevalence of HCV (67%) with a predominance of subtype 4a (55%) [2]. Chronic C hepatitis (CHC) associated with increased risk for cirrhosis and hepatocellular cancer [3]. there is an alarming increase in the incidence of HCC in HCV patients in Egypt [4]. HCV infection is one of the major causes of morbidity and mortality. It is responsible for inflammation and the formation of scar tissue (fibrosis) by release of free radicals e.g., hydroxyl radicals [OH], superoxide anion radical [O·2], hydrogen peroxide [H2O2] and nitric oxide [NO] that leads to specific oxidation of some enzymes, protein oxidation and degradation [5]. Enzymatic antioxidant such as superoxide dismutase [SOD], glutathione peroxidase

[GPx] and catalase [6] eliminate their effects. In addition, nutritional and botanical treatment regimes, coupled with a healthy lifestyle, can improve the functioning of the immune system by increasing antioxidant levels in the body and thus minimize damaging effects of free radicals to liver cells [7]. Vitamin D is an osteroid hormone, which is mostly known as a regulator of calcium and bone metabolism. Additionally, Vitamin D has important non-skeletal effects which are involved in many biological processes [8]. Also, it has anti-inflammatory and anti-fibrotic effects. Vitamin D occurs as vitamin D3 (25(OH)D3; cholecalciferol) and vitamin D2 (25(OH)D2; ergocalciferol). Serum 25(OH)D levels are inversely correlated with parathyroid hormone (PTH) levels. Sun exposure, seasonality, place of residence, diet and the extent of skin pigmentation affect vitamin D bioavailability, where, the main form of vitamin D, are produced in the skin by means of sunlight exposure, while the remainder is retrieved from dietary components [9,10). Vitamin D2 does not depend on sunlight and only minute amounts of vitamin D2 are derived from plants [11]. Low vitamin D serum levels are associated with many human diseases [12] and clearly associated with unfavorable clinical outcomes and accelerated progression of chronic liver diseases due to viral hepatitis, alcohol consumption and NAFLD [13,14,15,16]. Vitamin D deficiency may contribute to liver damage through increased inflammation and fibrosis may also contribute to reduced antiviral responses [17,18]. Several studies had documented the protective properties of vitamin D in preventing HBV and HCV replication and in retarding clinical progression of HBV/HCV-related liver diseases [15].

Nigella sativa (N. sativa), commonly known as black seeds, belongs to the botanical family of Ranunculaceae. N. sativa is used as a food condiment in the Middle East. Its seeds/oil has been shown to possess anti-inflammatory, antiviral and antioxidant effects [19,20]. Thymoquinone (TQ) is a chief bioactive constituent of N. sativa that holds promising pharmacological properties against several diseases. It exhibits outstanding antioxidant, anti-inflammatory, anticancer, and other important biological activities [21,22]. Green tea has various biological and pharmacological activities, including anti- inflammatory, antibacterial, antitumorigenic, and antiviral activities [23]. Green tea leaves exert inhibitory effects on HCV replication [24]. This Study demonstrated a correlation between herbal supplements of liver disease and Vitamin-D level in males and females. The study was conducted to elucidate the Vitamin-D-metabolism in chronic liver disease and its role as marker in determining the severity of the liver disease as a result to chronic active hepatitis C infection.

2. Materials and Methods

This This is study carried out at Tudor Bilharzia institute in Egypt from June 2018 to June 2020. The study was approved by the regional Ethics Committee of General Organization for Teaching Hospitals & Institutes (GOTHI) National Hepatology and Tropical Medicine. Research Institute (NHTMRI- REC) and informed consent was obtained from all the participants before enrolment. Forty-eight cirrhotic patients (24 males and 24 females with age range between 20 - 60 years) were selected from Tudor Bilhariz institute, and Viscera unit. The study criteria included all patients diagnosed with HCV and negative for HBV. Exclusion criteria included patients on IFN-α therapy; infection with HBV or hepatitis immunodeficiency virus; drug induced liver diseases; All patients met the diagnostic criteria of liver cirrhosis by clinical, biochemical, and ultrasonographic findings. The cause of liver dysfunction was hepatitis C. Twenty- two healthy individuals matched for sex and age were selected as control.

Table (1): Comparison between the studied groups (cirrhotic patients with and without herbal treatment and normal controls) regarding laboratory parameters.

	Controls (gr I) (n= 22)	Non-herbal treated cirrhotic (gr II) (n= 24)	Herbal treated cirrhotic (gr III) (n= 24)	P	P1	P2	P3
ALT (U/L)	16.14a ± 1.75	200.75b ± 85.7	144.33c ± 64.85	0.001	< 0.001	< 0.001	0.0134
AST (U/L)	19.30 a ± 4.56	149 b ± 50.5	103.75 c ± 27.53	< 0.001	< 0.001	< 0.001	0.0004
GGT (U/L)	12.85 a ± 3.29	79.18 b ± 4.67	61.6 b ± 17.2	0.001	< 0.001	< 0.001	< 0.001
ALP (U/L)	114.8 a ± 7.0	50.39 b ± 24.39	104.2 b ± 15.69	0.001	0.0056	< 0.001	< 0.001
Serum bilirubin (mg/dl)	0.77 ±0. 33	1.95 ± 0.22	1.59 ± 0.76	0.01 0.912	0.0001	0.0001	0.0001
Serum albumin (g/dl)	4.11 ± 1.02	2.72 ± 0.95	3.37 ± 0.48	0.047	0.0026	< 0.001	0.0044
PCR-HCV IU/ml	13.7 ± 0.16	452.13 ± 12.73	222.16± 12.16	< 0.001	< 0.001	< 0.001	< 0.001
GSH (mg/dl)	58.89 a ± 6.1	19.50 b ± 4	26.51 c ± 4.38	< 0.001	< 0.001	< 0.001	< 0.001
SOD (U/L)	5.40 a ± 1.70	1.70 b ± 0.41	2.91 c ± 0.37	0.001	0.001	0.001	0.001
MDA (mmol/l)	5.54 a ± 0.54	135.41 b ± 11.31	91.05 c ± 13.95	< 0.001	< 0.001	< 0.001	< 0.001
AFP (ng/ml)	8.5 a ± 0.54	300 b ± 53.31	16 c ± 3.5	< 0.001	< 0.001	< 0.001	< 0.001
25 OH-D3 (ng/ml)	33.90 a ± 8.04	12.92 b ± 2.75	19 c ± 2	< 0.001	< 0.001	< 0.001	< 0.001
PTH (pg/ml)	24.85 ± 7.23	14.66 ± 3.74	19.86 ± 5.2	0.01	< 0.001	< 0.001	0.0002

The study population was divided into three groups according to herbal treatment as following: Group I: 22 healthy normal person (11 males and 11 females), Group II: 24 cirrhotic patients didn't receive herbal treatment (12 males and 12 females) and Group III: 24 cirrhotic patients treated with Herbal mixture (12 males and 12 females). They received one spoonful of herbal mixture composed of equal volumes of mixed powder of fenugreek, Nigella sativa and green tea two times daily for 2 months. Blood samples were collected for laboratory investigations including: -Liver enzymes: alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) activities were measured according by Reitman and Frankel [25]. -HCV RNA using quantitative real time polymerase chain reaction (qRT-PCR) using Roche Amplicor HCV monitor version 2.0 (Roche Diagnostics, Branchburg, NJ) [26]. with lower detection limit < 50 IU/ml. Malondialdehyde (MDA) was carried out according to the method of Ohkawa and Yagik, [27]. Glutathione was carried out according to the method of Beutler et al., [28]. Superoxide dismutase activity was carried out according to the method of Nishikimi and Yogi, [29]. Parathyroid hormone (PTH) according to the method of Bouillonr,etal., [30]. Serum α-fetoprotein

(AFP) was assayed according to Forest and Pugeat [31], using kits purchased from VIDAS bio Merieux (France) -Vitamin D (25- hydroxyl cholecalciferol) according to the method of (SDS, PAGE) weber and Osborn., Bouillon, [32,33].

The statistical significance between means was calculated by Student's t- test, Mann-Whitney U test or analysis of variance (ANOVA). The difference is considered significant when $P < 0.05$.

3. Results

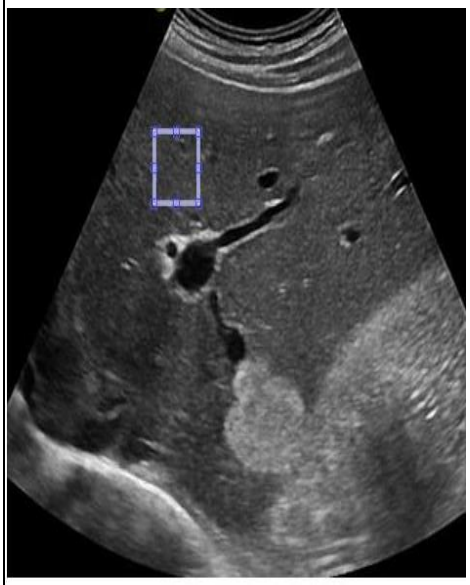
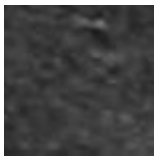
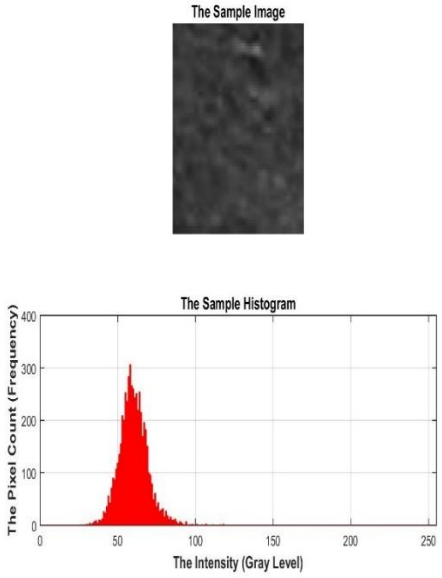
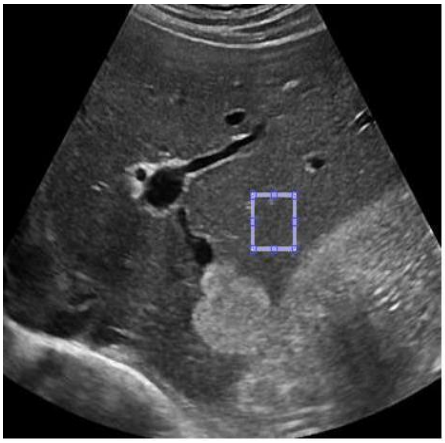
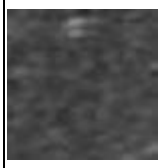
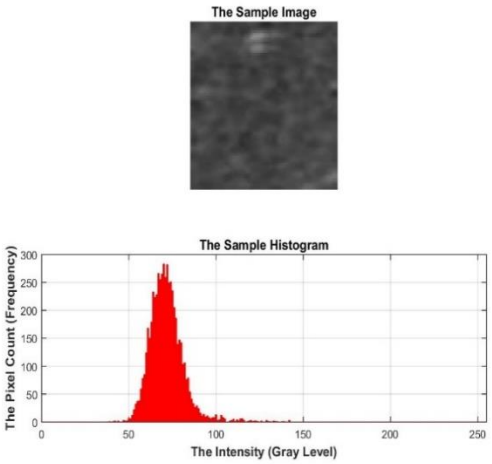
In our study, liver enzymes (ALT, AST, and GGT) and serum bilirubin were significantly decreased, and ALP and serum albumin levels were significantly increased in cirrhotic patients received herbal treatment (gr III) than those without treatment (gr II) (Table 1). We noticed that within gr III, herbal treatment was more effective in male patients than females regarding serum AST and ALT while more effective in females than males regarding ALP and γGT levels. However, herbal treatment had no significant effect on albumin and bilirubin levels between males and females' patients (gr III) (Table 2).

Table (2): Comparison between the studied groups (cirrhotic patients with and without herbal treatment and normal controls) regarding laboratory parameters.

Biochemical parameters	Herbal treated cirrhotic males (n= 8)	Herbal treated cirrhotic females (n= 8)	P-value
ALT (U/L)	118.45 ± 52.4	170.20 ± 77.3	0.003
AST (U/L)	100.5 ± 6.10	107 ± 2.7	0.11
GGT (U/L)	56.1 ± 17.7	67.10 ± 16.7	0.05
ALP (U/L)	100.40 ± 17.22	108.00 ± 14.16	0.09
Serum bilirubin (mg/dl)	1.51 ± 0.13	1. 66 ± 0.18	0.24
Serum albumin (g/dl)	3.32 ± 0.58	3.42 ± 0.38	0.63
PCR-HCV (IU/ML)	200,12±10	208,16±14	0. 01
GSH (mg/dl)	25.43 ± 3.74	27.58 ± 5.01	0.07
SOD (U/L)	2.97 ± 0.48	2.85 ± 0.25	0.25
MDA (mmol/l)	82.13 23.91	99.96 ± 3.98	0.04
AFP (ng/ml)	12 ±3.5	20 ± 3.5	0.001
25 OH-D3 (ng/ml)	20.00 ± 2.0	18.00 ± 2.00	0.22
PTH (pg/ml)	18.71 ± 1.21	21.0 ± 9.19	0.26

Mean Value	Standard Deviation	Coefficient of Variation	Maximum Value	Minimum Value	Skewness Value
93.78008889	13.12353067	0.139939414	132	57	-0.0985998
53.97617778	15.24879498	0.282509722	126	3	0.457694299

Table (3): Ultrasound for liver.

4. Discussion

The aim of this study is to investigate biochemical effect of herbal therapy on serum vitamin D and free radicals and antioxidants in patients with HCV-related liver cirrhosis (LC). The higher levels of ALT, AST and γGT are attributed to the viral pathological changes in hepatocytes which distorted the cellular permeability causing releasing of enzyme content. Raised bilirubin may be due to impaired hepatic secretion of bilirubin. Decreased ALP and albumin levels are owing to impaired hepatic synthetic function in cirrhotic patients. Barakat et al. [34] showed that N. sativa seed oil potentially alleviated serum liver enzymes in Egyptian HCV patients. Mallikarjuna et al. [35], also revealed that N. sativa oil administration was tolerable, safe, decreased viral load, and improved oxidative stress and clinical condition. We found that patients received herbal treatment (gr III) had significantly lower MDA levels compared to those didn't receive treatment (gr II) but these levels were still higher than that of control group (gr I). On the other hand, the herbal treated group (gr III) had significantly higher GSH and SOD levels than non-treated group (gr II), but lower than normal (gr 1) **(Table 1)**. We noticed that herbal treatment (gr III) was more effective in male patients than females regarding MDA and SOD levels while more effective in females than males regarding glutathione GSH levels **(Table 2)**. This result is agreed with Choi J and Ou JH., [36]. The reactive oxygen species (ROS) play an important role in the development and progression of inflammatory liver disease mediated by HCV Hepatitis C virus increased production of free oxidation radical which reflected on the obtained higher values of MDA and caused exhaustion of antioxidant activity [37]. However, Friedman et al., found that there were no significant differences between erythrocyte and plasma lipoperoxides in cirrhotic patients and controls. The levels of glutathione and superoxide dismutase were significantly decreased in cirrhotic patients [38]. Łukasz et al., observed that disorders in the structure of erythrocyte membrane proteins might be developed as a consequence of oxidative stress that was associated with an increase in membrane lipids fluidity. Increased fluidity of erythrocyte membrane may be a result of disorders in protein-lipid interaction or membrane lipid peroxidation activity [39]. In this study, AFP levels were significantly decreased in herbal treated patients (gr III) than non-treated patients (gr II) but still higher than controls (Table 1). In addition, herbal treatment (gr III) was more effective in male patients than females regarding AFP levels (**Table 2)**. Low grade elevation of AFP is seen in benign liver disease including acute and chronic hepatitis and cirrhosis, due to hepatic inflammation and viral replication [40]. Our study showed significant decrease in serum 25-hydroxycholecalciferol (25OH- D3) concentration in cirrhotic patients than control group. On the other hand, parathyroid hormone (PTH) concentration significantly decreased in diseased persons than control group **(Table 1)**. Moreover, serum 25OH-D3 concentrations were significantly increased and in herbal treated patients (gr III) than non-treated patients (gr II) but still lower than controls (Table 1). In addition, herbal treatment (gr III) was more effective in female patients than males regarding 25OH-D3 levels but without statistical significance, **(Table 2)**. Some authors have studied vitamin D metabolism in patients with HCV-related chronic liver disease compared them with healthy controls. They reported that serum 25OH-D3 levels decreased in those patients and this decrease was accentuated as the condition progressed toward cirrhosis. Hepatocellular dysfunction increases serum PTH levels as a result of vitamin D deficiency [41and42]. The attained improvement in liver function of patient received herbal treatment may be related to antioxidant effect besides

biological and pharmacological activities including anti-inflammatory, antibacterial, antitumorigenic, and antiviral of these herbs [43]. Green tea is widely consumed as a healthy beverage, and it has been considered as an alternative medicine for preventing cancer formation and infectious diseases owing to its diverse pharmacological effects [44]. Hoan et al., [45] reported that administration of compounds with antioxidant and immunomodulatory properties could be a reasonable strategy to halt the natural course of the disease e.g., reduction of hepatic fibrosis. Fenugreek (Trigonella foenum-graecum) is a medicinal herb with numerous health benefits. Fenugreek tea made from its seeds has been gaining popularity and may play a role in heart health, aid digestion, fight inflammation, and increase the levels of the antioxidant enzyme; glutathione [46]. *N. sativa* administration in HCV patients is safe and tolerable and results in a significant improvement in viral load, oxidative stress and laboratory markers. Moreover, the clinical improvement and better glycemic control in patients with diabetes indicate a potential role for N. sativa in improving the clinical outcome of HCV patients [47,48]. In conclusion, our findings suggest that administration of N. sativa, Fenugreek and green tea mixture to HCV-cirrhotic patients was safe, tolerable, decreased viral load, and improved liver function. In addition, the current study recommends the use of this herbal mixture to increase their synergetic effects against HCV and decrease their side effects.

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