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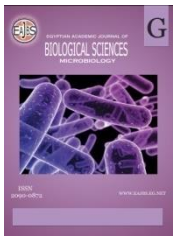

Deciphering The Correlation Between Triclosan and Immune Response to HCV Infection

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ABSTRACT

Triclosan has antibacterial, antifungal, and antiviral activities, and a low aqueous solubility but is highly soluble in organic solvents, also it has high lipophilicity. This study aimed to determine whether there is a correlation between the presence of triclosan in hepatitis C virus (HCV) patients' blood samples and illness exacerbation. This study was conducted on 44 patients who were confirmed as HCV infection positive only and did not receive any treatment for HCV infection at the Cardiac and Gastroenterology Center Hospital in Sohag, Egypt. From each patient plasma and serum samples were collected for quantitation of HCV titers using real-time PCR and HCV antibody titers using ELISA. While the amount of triclosan in representative 12 serum samples was measured in monograms by the GC-MS instrument. The results revealed that there is an inverse proportion between the triclosan and HCV particles, and a direct relationship between triclosan and HCV antibody titers. In conclusion, triclosan can confer antibody stimulation and antiviral action against HCV.

INTRODUCTION

Triclosan (TCS, 5-chloro-2-(2,4-dichloro phenoxy) phenol) is described as a broad-spectrum antimicrobial agent and has antibacterial and antifungal properties (Ciba Speciality Chemicals., 2001). TCS is a typical antibacterial compound present in household cleaners, and other consumer goods (McMurry *et al.,* 1998). It is a bacteriostatic agent at small doses due to its harmful effect on bacterial enzymes responsible for the composition of the cell membrane and cell wall fatty acids. TCS interrupts the bacteria membrane at high concentrations, trying to kill it (Fahimipour *et al.,* 2018; Jing *et al.,* 2020). TCS, which was developed in 1972 as an antibacterial factor in a surgical scrub composition, has a similar structure to the chemical diphenyl ether group and is well-tolerated and safe. Since then, it has become one of the most common preservatives in a wide variety of products including cleaning agents, soap, shampoo, detergent, toothbrushes, rinses, and textiles for many consumers. Due to extensive environmental contamination and its identification in streams and wild animal bodily fluids, TCS usage was rapidly expanded in the last 20 years. (Rodricks *et al.,* 2010).

TCS is detoxified in the body through glucuronidation via UDP-glucanosyltransferases (Moss *et al.,* 2000). TCS has been shown to interfere with many hormones and function as a mild endocrine disruptor (Crofton *et al.,* 2007). According to human and animal research, TCS therapy produces prion inflammatory responses in liver fibrosis, and hepatic immunity decreases (Kita *et al.,* 2002; Seki *et al.,* 2009). TCS-treated mice had significantly more inflamed portal vein leukocytes (Cd45+) in their fibrogenic livers. TCS produced consistent amounts of chemokine receptor 2 (CXCR2) in rats. CXCR2 was related to histological liver abnormalities, tumor angiogenesis, and metastatic potential, and is associated to increase liver inflammation (Kim *et al.,* 2001; Kubo *et al.,* 2005). It has been used in skincare for over 30 years all over the world. In a correct dose, triclosan supplies fast and broad-spectrum antibacterial activity (Bhargava and Leonard., 1996). The antiviral activity of TCS against various viruses has been investigated in human and animal experiments at different concentrations and formulations (Jones et al., 2000; Lages et al., 2008; Dellanno *et al.,* 2009). Triclosan's safety as an active ingredient has been confirmed via neurotoxicity, toxic effects, mutagenicity and reproduction (DeSalva *et al.,* 1989).

Like triclosan, boron-containing compounds such as boromycin have shown antiviral properties against the HIV-1 strain. (Kohno *et al.,* 1996). In addition, another study has suggested that boronic acid has antiviral properties against the hepatitis C virus (HCV). (Trippier and McGuigan.,2010). Till now, no research has demonstrated the antiviral activity of textile materials treated with boron compounds.

TCS and Sodium pentaborate pentahydrate (SPP) were applied for the first time to cotton textile materials and showed antiviral effects against poliovirus type 1 (PV-1) and adenovirus type 5 (AV-5). Both virus titers were reduced by 60% using these cotton fabrics treated with 0.03% TCS and 3% SPP concentrations indicating its considerable effectiveness against enveloped and non-enveloped DNA viruses such as hepatitis B virus (HBV), and against enveloped and non-enveloped RNA viruses such as HIV, HCV, Ebola, middle east respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) (Iyigundogdu *et al.*,2017).

Triclosan may weaken the immune defense against infections or increase sensitivity to allergens and inflammatory disorders (Kuo *et al.,* 2012; Nowak *et al.*,2019). It has been found to increase the risk of atopic asthma and aeroallergen sensitization in children 6 -12 years old (Spanier *et al.,* 2014). The capacity of triclosan to enhance hypersensitivity disorders raises the possibility of immunological alterations, such as elevated CD4+ and CD8+ T-lymphocyte ratios, reduced mononuclear cell proliferation, and elevated autoantibody frequency (Rees Clayton *et al.,* 2011). Also, triclosan concentrations up to 10 μM could entirely suppress the apoptotic function of human natural killer cells (Udoji *et al.*,2010), a crucial defense mechanism of the innate immune system against cancer cells and virally infected cells (Orange, 2008). A positive correlation between urinary triclosan titers in men and the occurrence of antinuclear antibodies, a sign of autoimmune disorder processes has been reported (Dinse *et al.,* 2016).

Hepatitis is curable in the case of hepatitis C, treatable, and preventable. Around 325 million people worldwide have viral hepatitis B and C, which results in 1.4 million annual fatalities. In 2019, 58 million individuals worldwide have chronic hepatitis C infection, which accounts for about 400,000 annual mortalities, by 2030, WHO aims to completely eradicate viral hepatitis B and C as a threat to public health. Only 21% of 58 million people with chronic hepatitis C had already been diagnosed, and only 13% had gotten treatment. An estimated 3.26 million children and teenagers, who were 18 years old

or younger, had chronic HCV infection in 2018 (WHO 2019**).**

Around 170 million people worldwide are chronically infected with the hepatitis C virus (HCV). This equals 3% of the global population (WHO 2009). HCV is a single-stranded positive-sense RNA virus that belongs to *Flaviviridae* and transmits from person to person through infected blood (Alter, 1996). A cohort analysis of 25,700 patients for screening HCV revealed that 450patients are at high risk of HCV sequelae and in need to receive help from antiviral medication (Mallette *et al.,* 2008). Although HCV infection cannot be avoided by vaccination, people infected with HCV should be checked for hepatitis A and B vaccination because of the increased risk of morbidity and death associated with the co-infection of these viruses (Alter, 1996; Koff and Muir, 2008).

To detect HCV infection, diagnostic techniques including HCV antibody enzyme immunoassays, transgenic immunofluorescent analysis, and quantitative real-time RT-PCR for HCV RNA are available. An enzyme immunoassay could produce false positive outcomes when used in low-risk groups, so a confirmatory test should be conducted after a positive enzyme immunoassay (National Institutes of Health, 2002). The recombinant immunoblot test detects antibodies to distinct HCV antigens more specific than just a positive enzyme immunoassay (Scott and Gretch, 2007). The HCV antibody detection test based on saliva may be available shortly (González *et al.*, 2008).

This study aimed to elucidate the effect of triclosan exposure on the activity of the virus and antiviral immunity production in infected patients and to figure out the correlation between the presence of triclosan in HCV patients and illness exacerbation.

MATERIALS AND METHODS

Inclusion Criteria:

The included patient in the study should be: -

1. Willing and able to supply written informed consent.
2. Male or female, age ≥ 18 years.
3. Chronic HCV infection (≥ 6 months) documented by medical history or liver biopsy.
4. HCV genotype at screening not determined by the Central Laboratory. Any non-definitive.

Patients must have the following laboratory parameters at screening: -

1. Alanine aminotransferase (ALT) ≤10 × upper limits of normal (ULN).
2. Aspartate aminotransferase (AST) ≤10 × ULN.
3. Hemoglobin ≥12 g/dL for males, ≥11 g/dL for females.
4. Platelets ≥ 50,000/mm3.
5. International normalized ratio (INR) ≤1.5 × ULN unless the patient has known hemophilia or is stable on an anticoagulant regimen affecting INR.
6. Albumin ≥3g/dL.
7. Direct bilirubin ≤1.5 × ULN.

The historical result from prior participation in this study was acceptable, if applicable no prior exposure to any interferon, ribavirin (RBV), or other approved or experimental HCV-specific direct-acting antiviral agent.

Exclusion Criteria:

The excluded patient from the study participation. Patients with any of the following were not eligible for participation in the study:

1. Current or prior history of any of the following:

a. Clinical hepatic decompensation (i.e., ascites, encephalopathy, or variceal hemorrhage).

b. Clinically significant illness (other than HCV) or any other major medical disorder that may have interfered with patient treatment, assessment, or compliance with the protocol, or is currently under evaluation for a potentially clinically significant illness (other than HCV).

c. Gastrointestinal disorder or postoperative condition that could have interfered with the absorption of the study drug.

d. Solid organ transplantation.

e. Significant pulmonary disease, significant cardiac disease, or porphyria.
f. Psychiatric hospitalization, suicide attempt, and/or a period of disability because of their psychiatric illness within the last 5 years. Patients with psychiatric illness (without the prior mentioned conditions) that was well-controlled on a stable treatment regimen for at least 6 months before baseline/Day 1 or that had not required medication in the last 12 months were eligible.
g. Malignancy within the 5 years before the screening, except for specific cancers that are cured by surgical resection (e.g., basal cell skin cancer). Patients under evaluation for malignancy were not eligible.

Sample Collection:

The samples were collected from 44 HCV-infected patients and 3 healthy individuals at Cardiac and Gastroenterology Center Hospital, Sohag, Egypt. The whole blood was collected in sterilized sodium citrate-containing tubes from males and females with writing data including age, sex, source of TCS, degree of liver injury before receiving treatment, and special medical examinations (Table 1).

Determination of HCV by Real-Time PCR:

Serum specimens were collected from 44 HCV patients, each specimen was divided into three replicates and stored at -80°C within 2 h of collection, (Halfon et al., 1996). The HCV RNA was extracted from each sample using the mirVanaTM miRNA isolation kit (ThermoFisher, cat. no AM1560) according to the manufacturer's instructions. The cDNA synthesis was performed using the miScript PCR system (miScript II RT kit, Qiagen, cat. no. 218161) according to the manufacturer's instructions. The real-time PCR was conducted using the miScript PCR system (miScript SYBR Green PCR kit, Qiagen, cat. no. 218073) in a total volume of 25ul containing; 12.5ul 2xQuantiTect SYBR Green PCR master mix, 2.5 ul miScript Universal primer, 2.5ul 10x miScript primer assay, 2ul of the prepared cDNA and 5.5ul RNase-free water. After that dispense, the mixture into a 96-well plate, seal it with heat-sealing film, and centrifuge for 1 min at 1000 xg to remove bubbles. The thermal profile was initial activation at 95°C / 15 min followed by 40 cycles of denaturation at 94°C / 15 s, annealing at 55°C / 30 s, and extension at 70°C / 30 s.

Anti-HCV Assays:

Enzyme-linked immunosorbent assay (ELISA) (Spectrum diagnostics, Egypt) for determination of anti-HCV (Hepatitis C Virus Antibody IgG & IgM) in human serum or plasma is a two-step indirect method. In the first step, sample and recombinant HCV-coated microplate wells are combined. The anti-HCV IgG and IgM antibodies present in samples during incubation bind to the well-coated antigens. Unbound serum antibodies are removed during the washing step. After washing, in the second step, enzyme (Horseradish peroxidase) conjugate is added to the reaction mixture. The Anti-HCV IgG and IgM antibodies captured to the solid phase in the first step react within enzyme conjugate. Then a complex is generated among the solid phase, the Anti-HCV within the sample, and the anti-human in the enzyme conjugate by anti-human immunoglobulin immunological reactions. After a second washing to remove unbound conjugate, substrate 3,3,5,5 tetramethylbenzidine (TMB) solution is added resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance at 450nm. The color intensity is proportional to the amount of Anti-HCV in the sample (Alter *et al.,* 1980; Houghton *et al.,* 1991).

Positive Control (polyclonal antibodies to HCV: core NS3, NS4, and NS5 conjugated to human IgG). The mean absorbance of Positive Control is equal to or higher than 0.5. Negative Control (human serum non-reactive for HBsAg and antibodies for HIV-1, HIV-2, and HCV). The mean absorbance of Negative Control is lower than 0.2 Note: -

The test is considered not valid if the mean absorbance of the negative control is

more than 0.2 and samples must be retested. Each plate must be considered separately when calculating and interpreting the results of the assay.

Extraction of Triclosan:

A representative 12 blood samples (3–5 mL) were centrifuged for 30 min at 2000×g. Serum samples (0.05–0.4 mL) were centrifuged for 30 min after being transferred to separate centrifuge tubes having 5 mL ethanol to sediment protein clumps. The deproteinized samples were then extracted twice with 6–20 mL dichloromethane. The organic phase was recovered and evaporated under nitrogen at a temperature lower than 37°C. Before LC analysis, samples were regenerated in 1 mL methanol, vortexed, and filtered using 0.45 um PVDF membrane filters.

Estimation of Triclosan Concentration in Plasma in Nanogram:

A high-performance liquid chromatography, a Shimadzu model LC-10 ADvp pump, a Rheodyne 7125 injection valve, and a 20-lL sample loop were utilized, as well as a PARC 400 electrochemical detector from EG & G (Princeton, NJ, USA) and a Hitachi L-7420 spectrophotometric detector (Hitachi, Japan) at Nawah Scientific Lab., Almokattam Mall, Street 9, 2nd floor, Al-Asmarat, Almokattam, Cairo, Egypt. A confined counterflow cell with an Ag/AgCl/0.1 M KCL reference electrode, a stainless-steel auxiliary electrode, and a dual glassy-carbon electrode for triclosan detection was used. Differential pulse voltammetric measurements were done using a KO269A Faraday cage and an EG and G Princeton Applied Research model 394 linked to an EG and G 325 Faraday cage with Smart stir. For solvents and analytes, 0.45-um cellulose acetate and polyvinylidene fluoride (PVDF) syringes and membrane filters were used, respectively. A Data Integrator for SISC Chromatograms.

Construction of Detector:

A length (8 cm) of Teflon tubing (1/32-inch i.d., 1/16-inch o.d.) was used to fabricate the voltammetric detector in a method as described by (Wang and Chu, 2004). The voltammetric detector for liquid chromatography: A strand of carbon fibers (from Formosa Synthetic Fiber Research Institute, polyacrylonitrile, PAN type, diameter 0.16–0.32 ml) was placed into one end of the Teflon tube and sealed with acrylic glue (obtained from Struers). To connect the Teflon tube to the fiber, a tiny copper wire was placed into the opposite end. The Teflon tube was then connected in series with the platinum wire, which served as a counter-electrode, and the Ag/AgCl reference electrode. The cell components were taped to an insulated plastic box for further stability. The eluate is supplied to the carbon-fiber electrode, which is put in an overflowing tank with a counter-electrode and reference electrode, that like a coulometric detector.

GC–MS/MS Analysis:

The chemical composition of samples was performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 250 °C held for 2 min, increased to the final temperature of 300°C by 30°C /min, and hold for 2 min. The injector and MS transfer line temperatures were kept at 270°C and 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral databases.

Statistical Study:

- Comparing between HCV patients and healthy humans in age, virus titration, antibodies titers, and triclosan estimation in blood samples. Table 3. We used SPSS V20 Statistical in the calculation P-value

range *, **, significant at 5% and 1% levels. An Independent t-test was used to compare the means among groups for all collected samples.

- Average of HCV patients and healthy human control in males and females one-way AOVA were used to compare the means among groups table 4.
- Average of HCV patients and healthy humans at different ages Table 5 one-way AOVA was used to compare the means among groups.
- Correlations of Anti-body quantities Index 0.9-1.1, and Triclosan by ng/ml table 6.

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

RESULTS AND DISCUSSION

In this study, HCV antibodies were estimated in 44 patients, their average ages were 49.5 (18-81) years, The number of males was represented as 32/44 (72.72%) and the number of females was 12/44 (27.27%) and three healthy people have not received any type of treatment for HCV and have an average age of 40.66 (24 – 55) years, 66.66% (2/3) males and 33.3% (1/3) females as control by the laboratory ELISA method like EIA in diagnostic laboratories. The results showed extremely low antibodies ranging from 1.3 to 21.95 ng/ml (Table 1), and these estimated antibody technique results agree with those obtained by third-generation EIAs tests which determine anti-HCV antibodies by using antigens from the surrounding core of HCV (NS3, NS4, and NS5 sites) in patients who suffer from chronic hepatic diseases (Colin *et al.,* 2001). We used instruments in this study that like the earlier studies were found to have a sensitivity of 98.9% and a specificity of 100%. As a result, while EIAs are distinguished by their simple use and low cost, this assay was also completely automated and proper for wide-range testing. Overall, EIAs were recommended for the determination of anti-HCV antibodies for HCV infections (Chevaliez and Pawlotsky 2008).

In this study, the amount of virus in each sample was estimated. The highest five samples showing the highest antigen titers were 3, 11, 15, 16, and 34 with virus quantities 5805405 – 9507934 IU/ml and antibody quantities 3.2 – 10.8 ng/ml (Table 1). The last four samples recorded the lowest virus quantities by the real-time PCR technique were 9, 10, 25, and 33 with virus quantities 10392 – 44264 IU/ml and antibody quantities 10.34 – 17 ng/ml (Table 1), which agreed with these studies techniques' amplification target (RT-PCR or TMA), can be used to quantify HCV RNA (bDNA assay). There are also many FDA-established quantitative assays for detecting HCV RNA (Chevaliez and Pawlotsky, 2012; Albertoni *et al.,* 2014; Mack *et al.,* 2012). In clinical practice, real-time PCR was the chosen technique for estimating HCV RNA levels. This assay was tremendously sensitive, had a large dynamic average of quantification, and could resist contamination from spreading. This result revealed the presence of an inverse relationship between HCV RNA levels and antibody production (Table 1) and supported reports stating that anti-HCV titer plays a vital role in expecting HCV viremia and appeared to be more clinically useful in patients who were also infected with HBV. In patients with an anti-HCV titer of more than 9, the patients were HCV-viremic. In special populations, however, all patients were HCV-non-viremic if their anti-HCV titer were less than 9, showing that anti-HCV could be used as a surrogate for active HCV infection even though we now have direct-acting antivirals for HCV treatment (Chen *et al.,* 2021; Huang *et al.,* 2019).

To know the occurrence of TCS and its concentrations in the blood HCV infected and healthy people and its correlation to anti-HCV antibody and virus quantities, TCS was estimated in randomly selected HCV-infected samples with various antibody and virus titers and healthy ones. The results should that TCS is present in all HCV-positive and healthy samples in, 9 HCV-infected patients and 3

healthy persons, but the measured amount of TCS was highly significant in HCV-infected persons in comparison to healthy persons as shown in Table 2 and Fig.1. The occurrence of TCS in healthy persons means triclosan penetrated the skin layer and reached the blood during using the TCS containing textiles (Soap, Shampoo, toothpaste, and deodorant). The presence of TCS in many human samples, such as urine, serum, and breast milk, in many earlier studies, reached 354 and 3,790 g/L, depending on how often TCS-containing products are used (Dinwiddie *et al.,* 2014). Several studies have been conducted on the applications of triclosan, which is present in the air, water, and land, and many products which are used in personal care, for example, soap and shampoo. Because this substance is active against microbes, especially fungi, bacteria, and viruses. (Bhargava and Leonard., 1996; Jones *et al.,* 2000; Lages *et al.,* 2008; Dellanno *et al.,* 2009)

In addition to that, earlier studies said humans and animals can absorb TCS through the skin, mucous membranes of the mouth cavity, or gastrointestinal tract routes and reach systemic circulation (Lin, 2000; Bagley and Lin, 2000; Moss *et al.,* 2000; Hovander *et al.* 2002; Queckenberg *et al.,* 2010; Fang *et al.,* 2016). In the current study, the results showed that in blood samples having the highest and the lowest antigens and antibodies values for HCV when antibodies increased the corresponding antigen value decreased (Tables 1 and 2). Also, a positive relationship between measured triclosan amount and antibody titers was noted (Fig. 2), where triclosan increased antibody production, so, may function as a stimulant substance for antibody production. This is harmony agreed with the study reported that humans exposed to pesticides have Endocrine disrupting chemicals (EDCs) (McConnachie and Zahalsky, 1992; Rosenberg *et al.,* 1999; Straube *et al.,* 1999; Ahmed, 2000). The data on HCV infection agreed with the reports which investigated whether the urinary concentration of Bisphenol A (BPA) in urine or triclosan was linked with allergies or assayed antibody titers in serum to a common herpesvirus pathogen, cytomegalovirus (CMV). Increased levels of CMV antibodies were thought to be a sign of a change in cell-mediated immunity (McDade et al., 2000; Koch *et al.,* 2006; Stowe *et al.,* 2007; Dowd and Aiello, 2004). These results showed that antibody production and triclosan have an inhibitory effect on the antigen HCV. This study is considered a critical recovery technique for interaction between these common substances such as EDCs, BPA, and triclosan with the human immune system. On the other hand, an inverse relationship between the amount of triclosan and quantities of HCV virus is shown in Table 2 and Fig. 3. In consequence of this relationship, an inverse relationship between quantities of anti-HCV antibodies produced and virus titers in the same sample indicates the ability of triclosan to stimulate the immune response and to function as an antiviral.

It worth noted that a case has high viremia (44264 IU/ml) with a high amount of triclosan (174.611ng/ml). It can be explained that HCV belongs to a specific genotype that resists triclosan. The most common states of microorganisms stayed in living cases in habitats, stationery, and biofilm cells, although exposed to 1000 mg/mL triclosan (2000 MIC), the same concentration that killed cells in log-phase. Cells still survived in biofilms and were resistant to triclosan (Junker and Hay, 2004).

Table 1. Information about HCV examined patients, TCS source, and HCV antibody and virus quantities.

Sample ID	Age (year)	Sex	Medications [a]	Source of TCS [b]	HCV Ab.	HCV Ab. quantities index(ng/ml)	Virus quantities (IU/ml)
1	70	M	NR	S	+ve	12.94	71400
2	39	F	NR	S-Sh	+ve	15.89	1358000
3	35	F	NR	S-Sh	+ve	7.25	5860242
4	38	M	NR	S-T	+ve	21.90	1731571
5	24	M	NR	S-T-D	+ve	21.95	1620000
6	22	M	NR	S-T-D	+ve	12.82	633395
7	28	M	NR	S-T-D	+ve	13.74	634692
8	22	F	NR	S-D-T	+ve	14.45	3050000
9	30	F	NR	S-D-T	+ve	12.77	44264
10	58	M	NR	S-T	+ve	10.34	10392
11	36	M	NR	S-T	+ve	12.15	5805405
12	20	F	NR	S	+ve	9.7	2015781
13	40	M	NR	S-T	+ve	5.96	2632414
14	56	M	NR	S-D-T	+ve	10.09	1968839
15	39	M	NR	S-Sh	+ve	10.8	6737373
16	42	M	NR	S-T-Sh	+ve	10.7	6821874
17	42	M	NR	S-D-T	+ve	11.84	654871
18	55	F	NR	S-T-Sh	+ve	11.22	672382
19	42	F	NR	S-Sh-T-D	+ve	8.1	2377582
20	18	F	NR	S-T	+ve	8.5	2427687
21	65	M	NR	S-T	+ve	12.1	599919
22	61	M	NR	S-T-D	+ve	13.2	756148
23	35	M	NR	S-Sh	+ve	14.2	855405
24	65	M	NR	S-T	+ve	1.8	476000
25	51	F	NR	S-Sh-T-D	+ve	17	15000
26	52	M	NR	S-Sh	+ve	14.6	955000
27	65	M	NR	S-T	+ve	9.5	1592000
28	66	M	NR	S-T-D	+ve	6.3	4915000
29	23	M	NR	S-D-T	+ve	12.0	173000
30	39	M	NR	S-T-D	+ve	10.1	1476532
31	25	F	NR	S-T	+ve	13.0	789875
32	61	M	NR	S-T-D	+ve	6.2	4864029
33	28	M	NR	S-T	+ve	15.4	23418
34	55	M	NR	S-T-D	+ve	3.2	9507934
35	35	M	NR	S-Sh	+ve	11.2	320076
36	81	F	NR	S-Sh-T	+ve	7.8	178945
37	63	F	NR	Sh- T-D	+ve	9.1	1096457
38	27	M	NR	Sh- T-D	+ve	5.8	5680792
39	48	M	NR	Sh- T-D	+ve	9.8	648048
40	66	M	NR	Sh T-D	+ve	15.2	493772
41	58	M	NR	Sh- T-D	+ve	14.4	737115
42	64	M	NR	S-Sh	+ve	1.3	523237
43	45	F	NR	S-Sh	+ve	13.2	524812
44	33	F	NR	S- T-D-Sh	+ve	21.2	54551
C1	24	F	NR	T-S-D	-ve	-[c]	0
C2	55	M	NR	T-S-Sh	-ve	-	0
C3	43	M	NR	T-S-D	ve	-	0

[a] NR = not receive medications against HCV
[b] S=soap; Sh=shampoo; T=toothpaste; D=deodorant
[c] Normal HCV antibody quantity index (0.9 – 1.1 ng/ml).

Table 2. The relationship among TCS amount, HCV antibodies and virus quantities in randomly selected HCV patients' samples and health ones.

Sample ID	Age (year)	Sex	Medications [a]	Source of TCS [b]	HCV family history	HCV Ab. quantities index(ng/ml)	Virus quantities (IU/ml)	TCS (ng/ml)
3	35	F	NR	S-Sh	NO	7.25	5860242	35.471
8	22	F	NR	S-D-T	NO	14.45	3050000	26.086
9	30	F	NR	S-D-T	NO	12.77	44264	174.611
10	58	M	NR	S-T	NO	10.34	10392	89.911
11	36	M	NR	S-T	NO	12.15	5805405	28.992
13	40	M	NR	S-T	NO	5.96	2632414	25.369
15	39	M	NR	S-Sh	NO	10.8	6737373	35.978
42	64	M	NR	S-Sh	NO	1.3	523237	27.059
C1	24	F	NR	T – S - D	NO	-	0	24.373
C2	55	M	NR	T – S - Sh	NO	-	0	22.643
C3	43	M	NR	T – S - D	NO	-	0	23.582

[a] NR = not receive medications against HCV
[b] S=soap; Sh=shampoo; T=toothpaste; D=deodorant
[c] Normal HCV antibody quantity index (0.9 – 1.1 ng/ml).

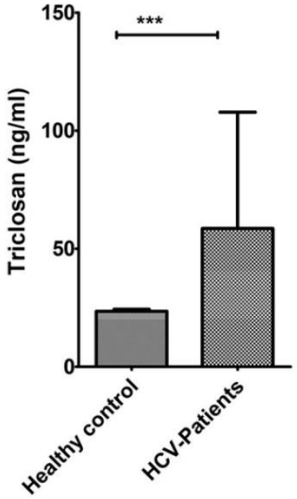

Fig. 1. Shown the relation between triclosan concentration and HCV infection where the amount of triclosan was highly significant related to virus occurrence in HCV infected persons in comparison to health persons at 0.0l value.

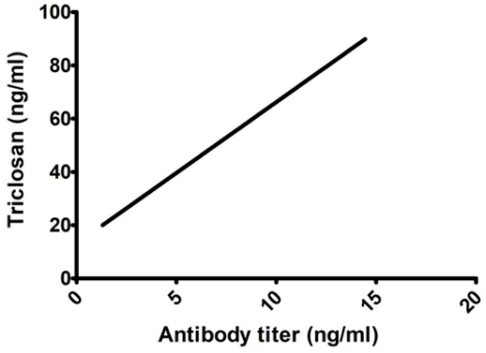

Fig. 2. The positive relation between triclosan concentration and ant-HCV antibody titers (ng/ml) where the amount of triclosan was significantly related to antibody in HCV infected persons in comparison to health persons at 0.05 value.

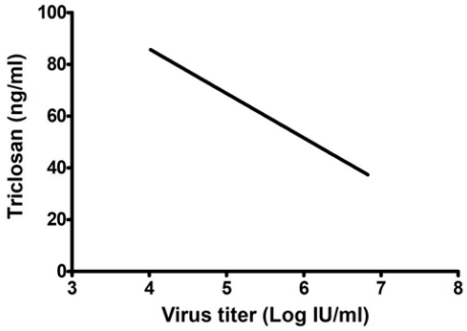

Fig. 3. The presence of inversed relationship between triclosan concentration (ng/ml) and HCV quantities (IU/ml) where the amount of triclosan was inversely highly significant related to virus quantities in HCV infected persons in comparison to health persons at 0.0l value.

Conclusion

The results of this research showed that there is a meaningful relationship between the estimated amount of triclosan HCV virus titers and anti-HCV antibody quantities. Where the triclosan occurrence is proportionally related to anti-HCV antibodies but inversely related to virus titers. This indicated its action as an immune stimulant and antiviral against HCV.

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Supplementary Index:

Table 1. Comparing between HCV patients and healthy humans in age, virus titration, antibodies titers, and triclosan estimation in blood samples.

		Age/years	Virus titration IU/ml	Anti-body quantities Index 0.9-1.1	Triclosan by ng/ml
Sample number 44	Minimum	18	10392	1.30	19.00
	Maximum	81	$9.51x10^6$	21.95	42.81
	Mean	44.70	$1.92x10^6$	11.38	26.87
	Std. Deviation	16.39	2.284E6	4.583	5.208
Control	Minimum	24	0	0	22.64
	Maximum	55	0	0	24.37
	Mean	40.67	0	0	23.53
	Std. Deviation	15.63	0	0	0.86
P-value		0.02*	0.00**	0.00**	0.00**

*, **, significant at 5% and 1% level.

Independent t-test was used to compare the means among groups

Table 2. Average of HCV patients and healthy human control in males and females.

	Sex	**Age/years**	Virus titration IU/ml	Anti-body quantities Index 0.9-1.1	Triclosan by ng/ml
Sample number 44	**Male**	46.93	$2.13x10^6$	11.05	27.30
	Female	39.93	$1.471x10^6$	12.08	25.97
Control	**Male**	49.00	0	0	23.11
	Female	24.00	0	0	24.37
P-value		0.01**	0.00**	0.00**	0.0*

*, **, significant at 5% and 1% level.

One-way AOVA **were used to compare the means among groups**

Table 3. Average of HCV patients and healthy humans at different ages.

	Age/years	Virus titration IU/ml	Anti-body quantities Index 0.9-1.1	Triclosan by Ng/ml
Sample number 44	<25	$1.653x10^6$	13.24	25.92
	25-30	$1.782x10^6$	11.99	26.86
	30-35	$0.0494x10^6$	16.99	24.28
	35-40	$2.008x10^6$	13.99	26.85
	40-45	$3.122x10^6$	9.15	28.13
	45-50	$0.586x10^6$	11.50	23.93
	50-55	$0.553x10^6$	15.80	23.77
	55>	$1.779x10^6$	9.043	27.28
Control	<25	0	0	24.37
	25-30	-	-	-
	30-35	-	-	-
	35-40	-	-	-
	40-45	0	0	23.58
	45-50	-	-	-
	50-55	-	-	-
	55>	0	0	22.64
P-value		0.00**	0.00**	0.02*

*, **, significant at 5% and 1% level.

One way ANOVA was **used to compare the means among groups**

Table 4. Correlations of Anti-body quantities Index 0.9-1.1, and Triclosan by ng/ml.

	Virus titration IU/ml	Anti-body quantities Index 0.9-1.1	Triclosan by ng/ml
Virus titration IU/ml	--	$-.384^{**}$	$.538^{**}$
Anti-body quantities Index 0.9-1.1		--	$-.310^{*}$
Triclosan by ng/ml			--

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level