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Colchicine as a Natural Toxicant

Stability-Indicating RP-HPLC Method

Highlights

Impact of Pharmacist Interventions

Management of Pulmonary Tuberculosis

Discovering Thoughts, Inventing Future

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Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in Tablet Formulation

By Deepthi R & Gowri Sankar D

Abstract- *Objective:* The present study aimed to develop a stability-indicating reverse-phase high performance-liquid chromatography (RP-HPLC) method for the estimation of Sofosbuvir, Velpatasvir, and Voxilaprevir in tablet dosage form and validated in accordance with ICH guidelines.

Methods: The optimized conditions for the developed RP-HPLC method are Agilent C18 (250 mm×4.6mm, 5μ) column maintained at 30°C with a mobile phase consisting of Buffer(0.1%OPA) and Acetonitrile taken in the ratio 55:45%v/v on isocratic mode at flow rate 1.0ml/min. The sample was detected at 220 nm.

Results: The retention time of Sofosbuvir, Velpatasvir, and Voxilaprevir was found to be 2.17, 2.731 and 3.55 min respectively. The developed method was validated for accuracy, precision, specificity, ruggedness, robustness and solution stability.

Keywords: *stability- indicating, method development, validation, RP-HPLC, sofosbuvir.*

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Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in Tablet Formulation

Deepthi R [α] & Gowri Sankar D [σ]

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Forced degradation studies were conducted by exposing the drug solution to various stress conditions such as acidic, basic, peroxide, neutral, photolytic and thermal conditions. The net degradation was found to be within the limits, indicating that the drug is stable in stressed conditions.

Conclusion: The developed method for the estimation of Sofosbuvir, Velpatasvir, and Voxilaprevir can be utilized for the routine analysis of the pharmaceutical dosage form.

Keywords: stability- indicating, method development, validation, RP-HPLC, sofosbuvir.

I. Introduction

Hepatitis C [1] is a liver infection which is caused by the Hepatitis C virus. The hepatitis C virus is a blood-borne virus and the most common modes of infection are through exposure to small quantities of infected blood. Globally, around 70 million people were suffering from Hepatitis C infection. Antiviral medicines like sofosbuvir, velpatasvir, and voxilaprevir, etc; can cure more than 95% of persons having Hepatitis C infection and reduce the causes of death.

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Sofosbuvir [2] (Fig 1) is a nucleotide prodrug and a hepatitis C virus (HCV) NS5B polymerase inhibitor with potential HCV inhibiting activity. Used as an antiviral drug in the treatment of Hepatitis C virus. It is chemically (S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4dioxo3,4 dihydropyrimidin1(2H)yl)4fluoro3hydroxy4methyltetrahyd rofuran2yl)methoxy)(phenoxy)phenylamino) propanoate.

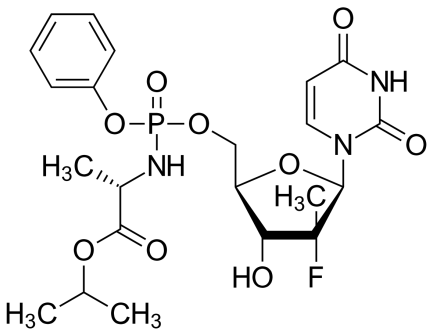


Figure 1: Structure of Sofosbuvir

Velpatasvir [3] (Fig 2) is a NS5A inhibitor which is used together with sofosbuvir to treat chronic Hepatitis C infection. Used as an antiviral drug in the treatment of Hepatitis C virus. Chemically it is methyl {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenylacetyl}-4-(methoxy methyl) pyrrolidin-2-yl]-1H-imidazol-4-yl}-1,11-dihydro[2] benzo-pyrano[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}carbamate.

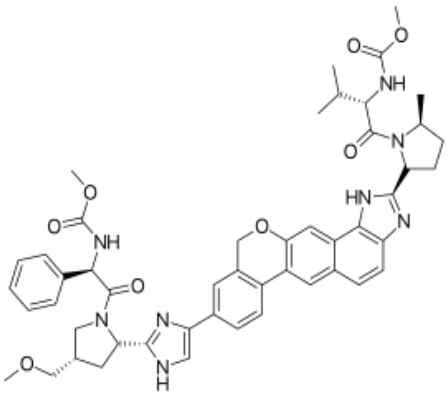


Figure 2: Structure of Velpatasvir

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Voxilaprevir [4] (Fig 3) is a protease inhibitor and acts as a transporter of polypeptide. Used as an antiviral drug in the treatment of Hepatitis C virus. chemically it is (1R,18R,20R,24S,27S,28S)N[(1R,2R) 2(Difluoromethyl)1{[(1methylcyclopropyl)sulfonyl]carba moyl}cyclopropyl]-28-ethyl-13,13-difluoro-7-methoxy-24 -(2-methyl-2-propanyl)-22,25-dioxo-2,21dioxa4,11,23, 26 -tetra aza penta cyclo nonacosa-3(12),4,6,8,10-pentaene-27-carboxamide.

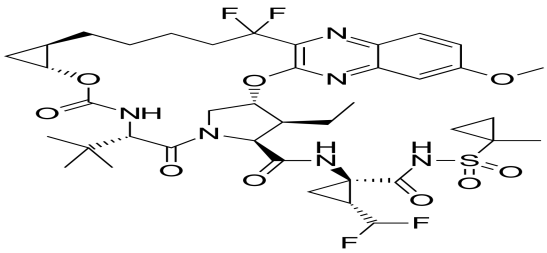


Figure 3: Structure of Voxilaprevir

As per the literature survey [5-11], it is learned that very few HPLC methods have been reported for the determination of Sofosbuvir, Velpatavir, and Voxilaprevir individually and in combination by HPLC but there is no method for stability-indicating and simultaneous estimation of all the three drugs.

Therefore, there is a need to develop a rapid and reliable Stability-indicating HPLC method for the simultaneous determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in bulk and pharmaceutical dosage form.

II. Materials and Methods

a) Reagents and chemicals

Sofosbuvir, Velpatasvir and Voxilaprevir working standards were procured from spectrum pharma research solutions, Hyderabad, as a gift sample. The VOSEVI tablets were supplied by the Medindia Pharma network. All the chemicals used were of AR grade purchased from Merck, Mumbai. All the solvents used were of HPLC grade purchased from Sigma-Aldrich, Mumbai.

b) Chromatographic conditions and Instruments

WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector, and an auto sampler integrated with Empower 2 software and Agilent C18 (250mm × 4.6mm, 5μ) column was used for the determination of Sofosbuvir, Velpatasvir, and Voxilaprevir. The optimized conditions included 0.1% Orthophosphoric acid (OPA) and acetonitrile (55:45%v/v) as mobile phase run on an isocratic mode at flow rate 1.0ml/min. The column was maintained at 300C and detection was done at 220nm. Other equipment used in the method was Ultrasonic bath sonicator (BVK Enterprises) and weighing balance (Denver).

c) Preparation of Diluent

A mixture of water and acetonitrile in the ratio of 50:50%v/v was used as diluents.

d) Preparation of Mobile phase

A mixture of 0.1% orthophosphoric acid and Acetonitrile in the ratio (55:45%v/v) was used as the mobile phase.

e) Preparation of Standard and Sample solutions

20mg, 5mg & 5mg of Sofosbuvir, Velpatasvir and Voxilaprevir working Standards were transferred to 50ml of volumetric flasks separately, 3/4th of diluents (as mentioned) was added to all the three flasks and subjected for sonication for 10 minutes. The final volume was made up with diluents to obtain a final concentration of 400μg/ml of Sofosbuvir, 100μg/ml of Velpatasvir, & 100μg/ml of Voxilaprevir respectively.

From the above stock solution, 1 ml was pipetted out into a 10ml volumetric flask and then the final volume was made with the same diluent. (40μg/ml of Sofosbuvir, 10μg/ml of Velpatasvir and 10μg/ml of Voxilaprevir respectively)

10 Tablets (Vosevi) were weighed accurately and the average weight was calculated. An amount equivalent of 1 tablet was collected into a 50ml volumetric flask; 15ml of diluents was mixed and sonicated for around 30 minutes. It was then subjected to making the volume with diluents.

Filtered the solution and diluted 1ml of the above solution to 10ml with diluents. (40μg/ml of Sofosbuvir & 10μg/ml of Velpatasvir & 10μg/ml of Voxilaprevir).

f) Method Validation

The developed method was validated in compliance with International Conference on Harmonization (ICH) guidelines [12, 13].

g) Specificity

The specificity of the method was determined by comparing the drug solution with the placebo solution and observed for the interference of placebo peak with drug peak.

h) Accuracy

The accuracy of the present method was determined by %recovery. The drug solution along with the sample was prepared in three concentration levels 50%, 100%, and 150%. Then the % recovery was calculated.

i) Precision

The precision of the method was estimated by injecting the six solutions of the standard into the HPLC system and the % relative standard deviation (%RSD) was calculated.

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j) Linearity

The linearity of the method was developed by preparing series of dilutions ranging from 12.5µg/ml - 75µg/ml for Bictegravir, 50µg/ml-300µg/ml for Emtricitabine and 6.25µg/ml-37.5µg/ml for Tenofovir alafenamide respectively and injecting them into HPLC system.

k) Ruggedness

Ruggedness was determined by injecting the six solutions of the standard into HPLC for different days. The % RSD was calculated.

l) Robustness

Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by ±5%, flow rate by ±0.1ml/min and column temperature by ±5°C.

m) LOD and LOQ

Calculation of limit of detection as well as Limit of quantification had been done by using standard Equations. LOD= 3.3×σ/S, LOQ =10×σ/S. Here σ denotes for the standard deviation of intercepts of regression lines, S denotes for slope.

n) Solution stability

Solution stability was estimated by analyzing the standard drug solution after storage for 24hrs under laboratory conditions.

o) Forced degradation studies

Forced degradation studies[14] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2N Hydrochloric acid for 30min at 60°C), basic (2N Sodium hydroxide for 30min at 60°C), Oxidation (refluxing the drug solution with 20%H2O2), neutral (refluxing the drug in water for 6h at 60°C), photolytic (exposing the drug solution to UV light by keeping the solution in UV chamber for 7 days or 200-watt hrs/m2 in photostability chamber), thermal (drug solution was placed in oven at 105°C for 6hrs) conditions.

III. Results

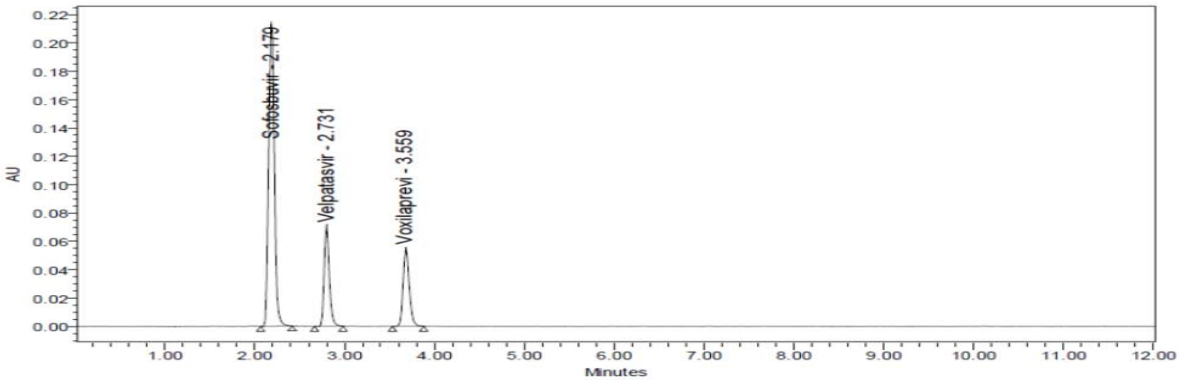


Figure 4: Optimized chromatogram of Sofosbuvir, Velpatasvir, and Voxilaprevir

Table 1: System suitability and validation parameter results

Parameter	Result		
	Sofosbuvir	Velpatasvir	Voxilaprevir
USP Plate count	4088	10175	126558
USP Tailing factor	1.0	1.2	1.2
USP Resolution	4.5	-	7.2
Precision (%RSD)	0.9	0.8	0.4
Accuracy	98.50-101.50	98.50-101.50	98.50-101.50
Specificity		Specific, No interference	
Linearity range (µg/ml)	10-60	2.5-15	2.5-15
Correlation coefficient, r^2	0.999	0.999	0.999
LOD (µg/ml)	0.11	0.10	0.06
LOQ (µg/ml)	0.34	0.29	0.17
Ruggedness (%RSD)			
Day1	0.9	0.8	0.4
Day2	0.9	1.9	0.6
Robustness (%RSD)			
Flow rate –	1.2	1.1	1.0

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Flow rate +	3.4	3.7	3.7
Column temperature –	2.8	2.7	2.9
Column temperature +	2.2	2.4	2.2
Mobile phase –	3.8	3.4	3.2
Mobile phase +	2.4	2.7	2.4
Solution stability (%RSD)			
(0 hrs)	1.0	0.6	1.0
(24 hrs)	0.9	1.0	0.6

Table 2: Forced degradation studies result

Stress condition	Sofosbuvir		Velpatasvir		Voxilaprevir	
	%Assay	%D	%Assay	%D	%Assay	%D
Acid	94.23	5.77	94.04	5.96	94.14	5.86
Base	95.35	4.65	95.10	4.9	94.49	5.51
Neutral	99.37	0.63	99.51	0.49	99.67	0.33
Peroxide	95.95	4.05	95.58	4.42	96.61	3.39
Photolytic	97.73	2.27	97.58	2.42	99.08	0.92
Thermal	98.22	1.75	96.43	3.57	98.71	1.29

%D- Percentage Degradation

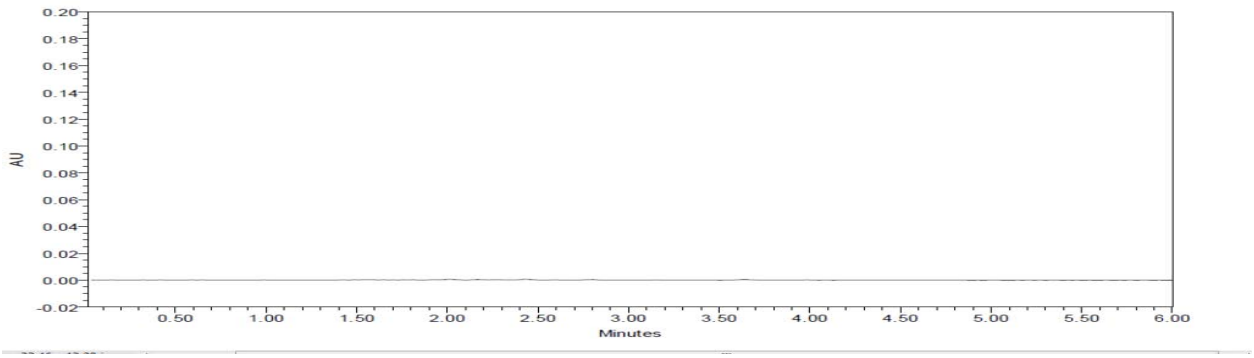


Figure 5: Blank chromatogram

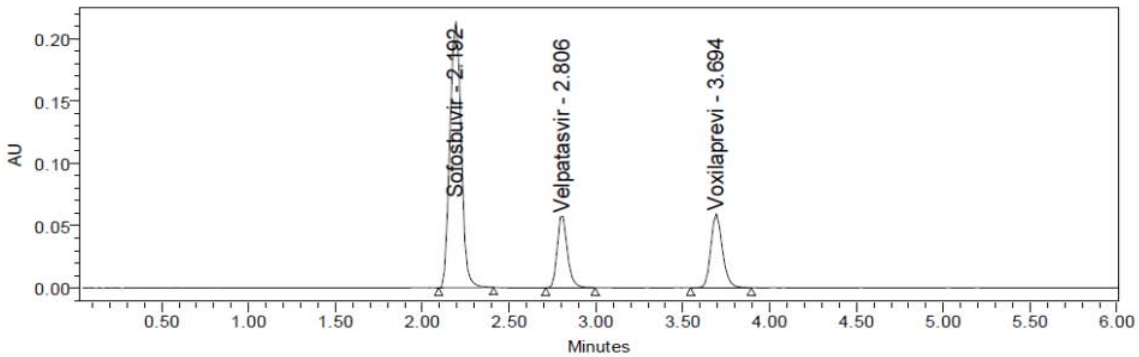


Figure 6: Standard chromatogram

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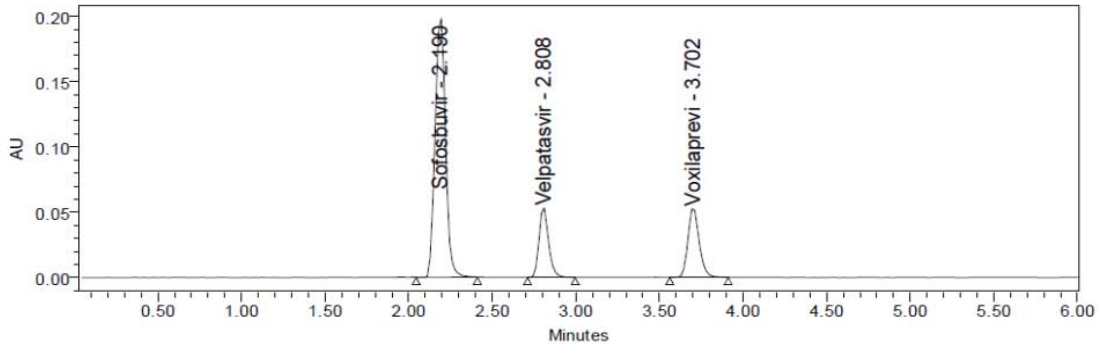


Figure 7: Sample chromatogram

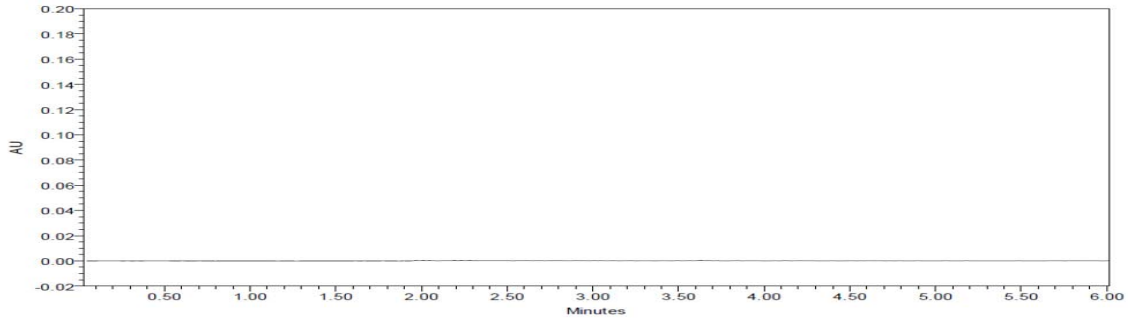


Figure 8: Placebo chromatogram

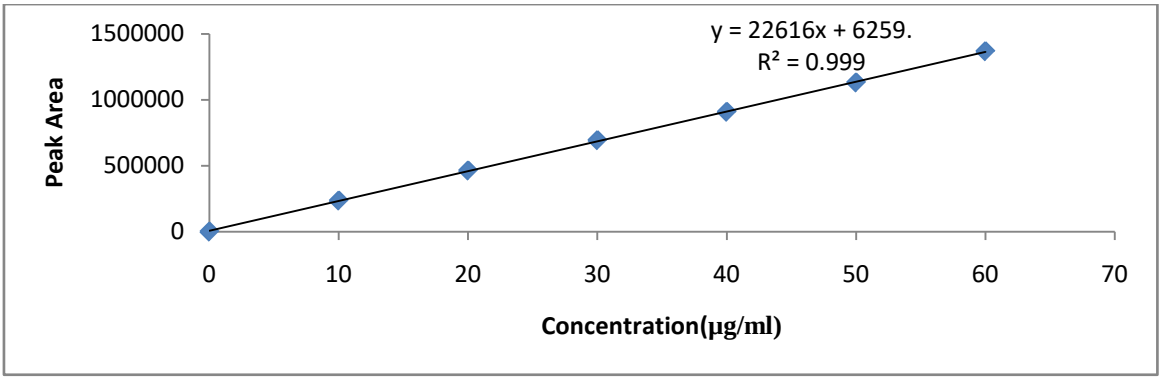


Figure 9: Linearity plot of Sofosbuvir

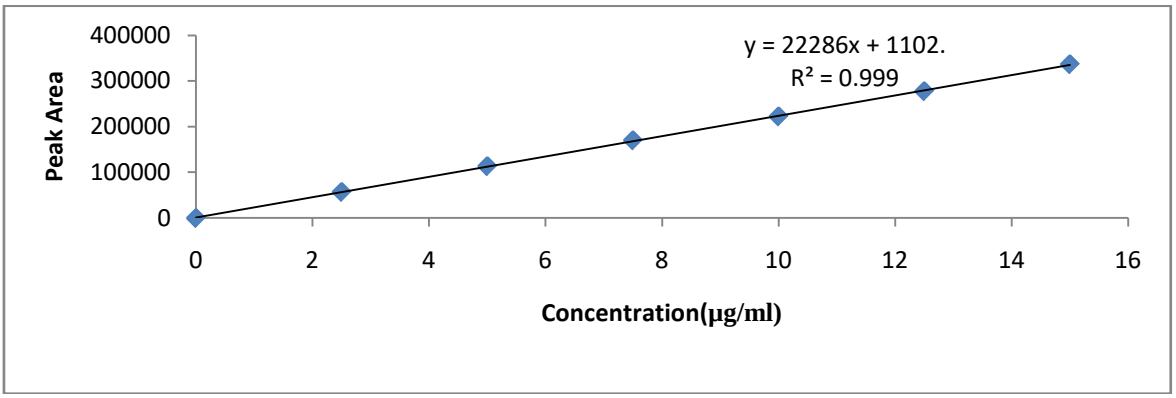


Figure 10: Linearity plot of Velpatasvir

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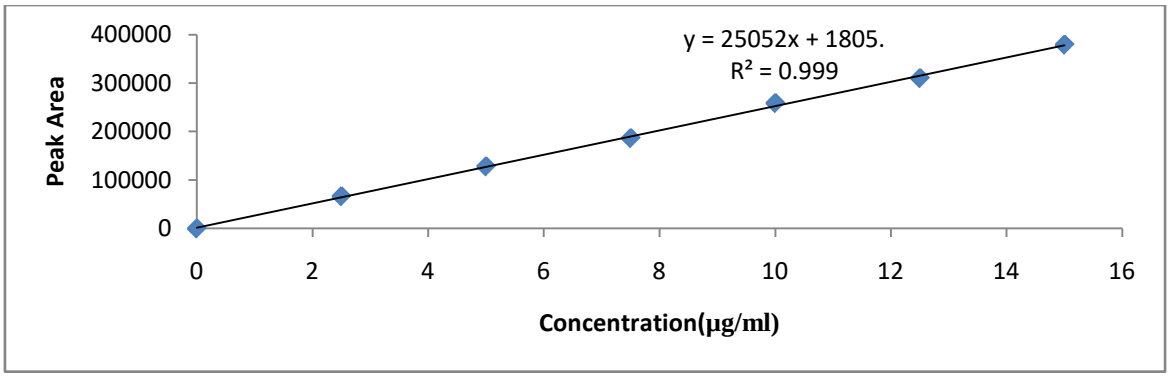


Figure 11: Linearity plot of Voxilaprevir

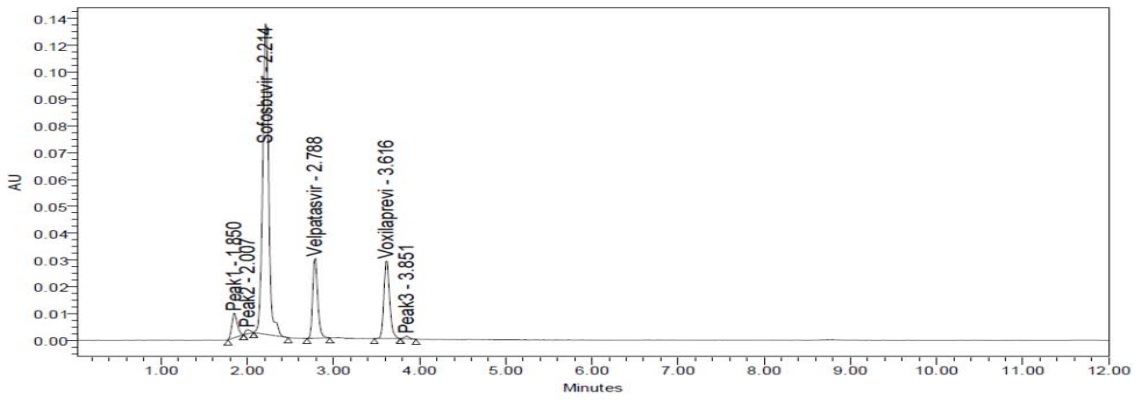


Figure 12: HPLC Chromatogram of Acid Degraded sample

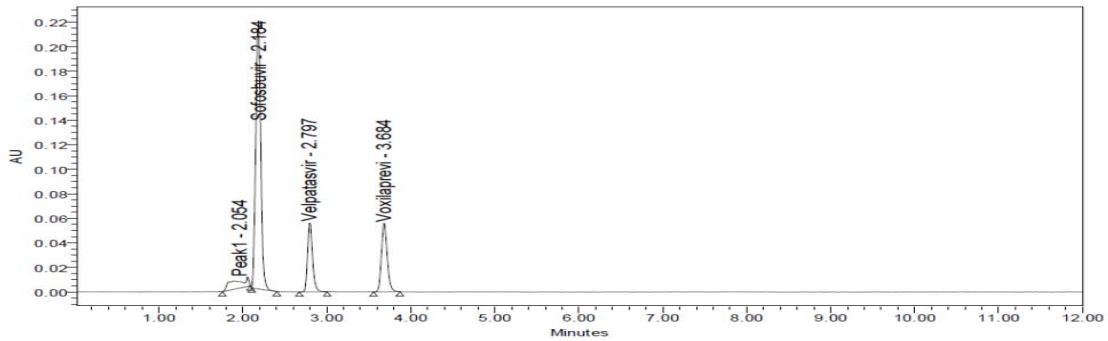


Figure 13: HPLC Chromatogram of Alkali Degraded sample

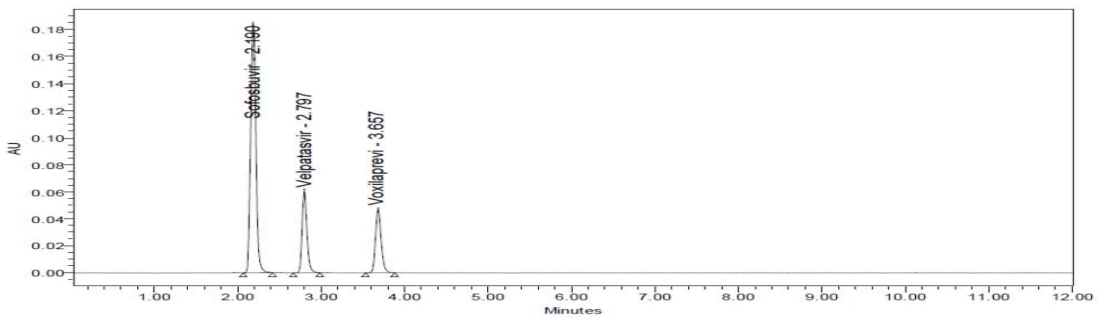


Figure 14: HPLC Chromatogram of Peroxide Degraded sample

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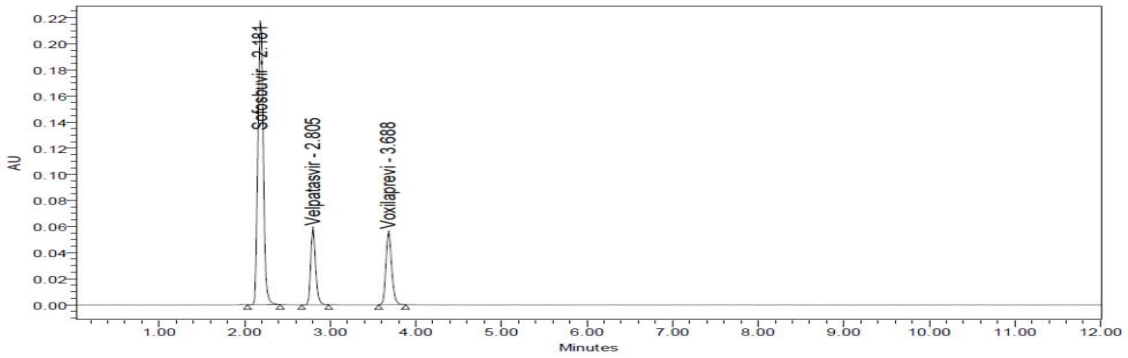


Figure 15: HPLC Chromatogram of Thermal Degraded sample

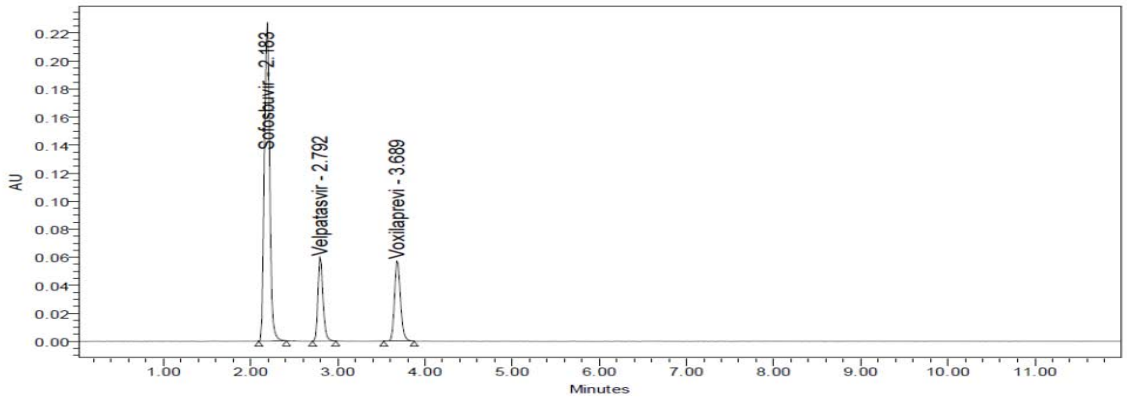


Figure 16: HPLC Chromatogram of UV Degraded sample

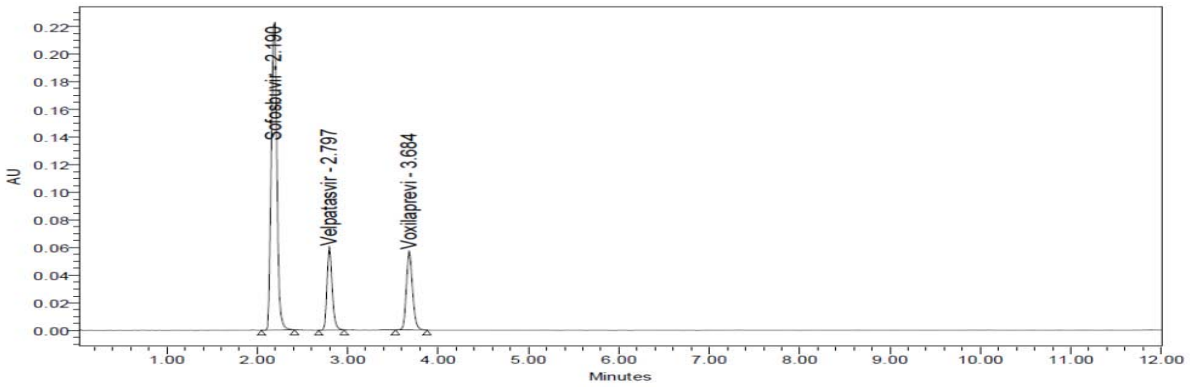


Figure 17: HPLC Chromatogram of Neutral Degraded sample

IV. Discussion

For the development of a new method for the simultaneous estimation of Sofosbuvir, Velpatasvir, and Voxilaprevir in bulk and pharmaceutical dosage form initially many mobile phases and many columns were tried to elute the drug peak with less tailing factor, more plate count and more resolution.

Waters HPLC Agilent C18 (250mm × 4.6mm, 5μ) column and Buffer: Acetonitrile (55:45%v/v) as mobile phase were selected based on peak parameters. The detection wavelength was found to be 220nm.

Prepared standard solution, sample solution, and blank solution were injected into the HPLC system and system suitability parameters were noted as summarized in Table 1 along with chromatograms as shown in fig. 4, 5, 6 and 7 respectively.

The developed method was found to obey Beer's law in the concentration range of 10-60 μg/ml for Sofosbuvir, 2.5-15 μg/ml for velpatasvir and 2.5-15 μg/ml for Voxilaprevir with a correlation coefficient of 0.999 each respectively. A linear graph was plotted between concentration and peak area as shown in fig.

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9.10 and 11 respectively and results are summarized in Table 1.

The method was found to be accurate as the % recovery was 98.50%-101.50% for all the three drugs and was within the limits. The %RSD was found to be less than 1 for all the three drugs indicates that the method was precise. The method was found to be specific, as there is no interference of retention time of placebo peak with that of drug peak. The placebo chromatogram was shown as fig. 8.

Forced degradation studies results indicate that the drug was found to be stable in various stress conditions as net degradation was found to be within the limits. The chromatograms were shown in fig. 12-17 and results were summarized in Table 2.

V. Conclusion

A specific, precise, stability-indicating method was developed for the determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in pure and tablet dosage form using RP-HPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate, specific and robust. From the degradation, studies conducted it is concluded that Sofosbuvir, Velpatasvir, and Voxilaprevir were stable at high concentrations of Acid, Base, Peroxide, Thermal, UV and Water stress study conditions. The run time was 5min which enables rapid quantitation of many samples in routine and quality control analysis of tablet formulations.

Acknowledgment

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Conflict of Interests

The authors claim that they have no conflict of interest. It has not meant to publish elsewhere. Moreover, it has not meant simultaneously presented for publication elsewhere.

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Impact of Pharmacist Interventions on Direct-Acting Antivirals Sustained Virologic Response and Drug-Drug Interactions

By Marcel Nogueira

Introduction- Infection by HCV is a growing global concern, given its effect on the mortality rate [1]. It is an important cause of cirrhosis, hepatocellular carcinoma, and liver transplant [2, 3]. In 2015, 71 million people were living with HCV. Several studies have shown that the number of new cases declined from the second half of the twentieth century. However, the incidence rate in 2015 was 23.7% (1.75 million new infections by HCV). This increase is related to different mechanisms of transmission. Besides the growing number of young injecting drug users in rural areas, there are reports of HCV transmission among men who have sex with men (MSM) infected with HIV. [4]. In 2016, the World Health Organization (WHO) showed overall goals for the elimination of HCV infection by 2030. This include a 90% reduction in new cases of chronic hepatitis C, a reduction of 65% of deaths, and treatment of 80% of eligible patients[5]. The old therapy in chronic hepatitis C has been a challenge because of the adverse events related to the use of oral ribavirin (RBV) and subcutaneous administration of peginterferon (PEG-IFN). This old therapy had low rates of SVR. In 2015, direct-acting antivirals (DAAs) were incorporated in Brazil. DAAs shows a better efficacy and safety profile, and has a better tolerability for patients [6].

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Impact of Pharmacist Interventions on Direct-Acting Antivirals Sustained Virologic Response and Drug-Drug Interactions

Marcel Nogueira

I. Introduction

Infection by HCV is a growing global concern, given its effect on the mortality rate [1]. It is an important cause of cirrhosis, hepatocellular carcinoma, and liver transplant [2, 3]. In 2015, 71 million people were living with HCV. Several studies have shown that the number of new cases declined from the second half of the twentieth century. However, the incidence rate in 2015 was 23.7% (1.75 million new infections by HCV). This increase is related to different mechanisms of transmission. Besides the growing number of young injecting drug users in rural areas, there are reports of HCV transmission among men who have sex with men (MSM) infected with HIV. [4]. In 2016, the World Health Organization (WHO) showed overall goals for the elimination of HCV infection by 2030. This include a 90% reduction in new cases of chronic hepatitis C, a reduction of 65% of deaths, and treatment of 80% of eligible patients[5]. The old therapy in chronic hepatitis C has been a challenge because of the adverse events related to the use of oral ribavirin (RBV) and subcutaneous administration of peginterferon (PEG-IFN). This old therapy had low rates of SVR. In 2015, direct-acting antivirals (DAAs) were incorporated in Brazil. DAAs shows a better efficacy and safety profile, and has a better tolerability for patients [6]. The Brazilian Ministry of Health has issued a protocol with the criteria for eligible patients and guidelines for the treatment of chronic hepatitis C. DAAs such as sofosbuvir (SOF), daclatasvir (DCV) and simeprevir (SMV) have been made available. In the second half of 2017, 3D (ombitasvir/paritaprevir/ritonavir+dasabuvir)(OBV/PTV/r +DSV) were included into this protocol [7]. Despite the aforementioned benefits over the old therapy, DAAs therapy presents a high risk of drug-drug interactions (DDIs)[7], [8]and there are some contraindications for all DAAs regimens [9]. The use of cytochrome P450 (CYP)/P-glycoprotein (P-GP) inducers (such as carbamazepine and phenytoin) are contraindicated, because of the risk of reduced concentrations of DAAs and high risk of virological failure [9]. Thus, it is essential to evaluate the continuous-use medication before starting treatment. DAAs have interactions with many drugs, especially in HCV-HIV co-infected patients in antiretroviral therapy [7]. CYP3A4 is the metabolic pathway for protease inhibitors such as SMV and NS5A inhibitor (DCV). These drugs can interact with enzyme inhibitors such as ketoconazole [10], [11], and inducers of CYP3A4, such as dipyrone and phenobarbital [12]. Similarly, daclatasvir (DCV) acts as a substrate and an inhibitor of P-glycoprotein (P-GP). Moreover, DCV is a weak inhibitor of organic anion transporters (OAT1B1/OATP1B3) and breast cancer resistance protein (BRCP) [11]. Sofosbuvir (SOF) is less involved in this, but it is as P-GP substrate and concomitant use of P-GP inducers should be avoided [10].

As a specialist in the management of pharmacotherapy, clinical pharmacist contributes to patient care by promoting the rational use of drugs and providing pharmacotherapy services [13]. A clinical pharmacist can identify cases of medication nonadherence, and provides support to hepatologists, optimizing patient care [14]. As well as encourages prevention measures, contributes to the reduction of HCV transmission, increases adherence to treatment and monitors adverse reactions [15]. Thereby, patient understands risks and benefits of pharmacotherapy, improving adherence and treatment outcome[16]. The involvement of the clinical pharmacist is beneficial forhepatology team because DDIs are a common event in the treatment of chronic hepatitis C. The identification and management of this is an intensive resource that requires adjustments to pharmacotherapy, in addition to continuous monitoring of patients. The assessment of DDIs in DAAs therapy and pharmacist interventions was recently published in the scientific literature [15], [17]. Nevertheless, in these studies, it was unclear whether the medical staff approvesthe pharmacist interventions.

II. Aim of the Study

Our primary objective was to evaluate the impact of pharmacist interventions related to DDIs on SVR. As secondary objectives: 1) to quantify DDIs identified by drug class and drug interaction potential; 2) to quantify pharmacist interventions recommended to medical staff and patient.

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III. Ethics Approval

Research Ethics Committee (Plataforma Brasil - protocol number 81497617.1.0000.0068) approved this retrospective study conducted under the STROBE Initiative. Informedconsentwasnotethicallyrequired for thisresearch.

IV. Method

a) Participants

We included patients with chronic hepatitis C, with DAAs prescription (SOF/DCV/SMV) with or without RBV or PEG-IFN, that received medication counseling by the Clinical Pharmacy of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP). We excluded patients who died, who had DAAs therapy suspended or without the final hepatitis C virus RNA-polymerase chain reaction test(HCV RNA-PCR).

b) Setting

We assessed data tabulated in Microsoft Excel between December 2015 and June 2017, collected from patients of infectious disease, liver transplantation, and gastroenterology outpatient services of HCFMUSP, a public tertiary teaching hospital. Before starting DAAs therapy, all patients were referred for Clinical Pharmacy of HCFMUSP and received medication counseling. This service promotes the rational use of medicines, patient care, and recommends conducts for medical staff to optimize pharmacotherapy. Concomitant use of drugs was analyzed by the electronic prescription system or by manual prescriptions. All included patients have received medication counseling by Clinical Pharmacy as established by the following steps: 1) individual or group counseling supported by an information leaflet that addresses issues such as chronic hepatitis C, HCV transmission, prevention, medication, adherence and patient care during DAAs therapy; 2) DDIs analysis on the HEP Drugs Interactions [8] and as necessary, pharmacist intervention addressed to medical staff, for management of DDIs; 3) Individualized guidance to facilitate medication administration times, according to routine of each patient; 4) tabulation of baseline characteristics, DDIs and pharmacist interventions on the database. By identifying DDIs, Clinical Pharmacy staff performed management of DDIs according to the clinical experience of each pharmacist and severity of interaction. Discussions were conducted with medical staff to solve this, in addition to sending letters when face-to-face contact was not possible. 5) DAAs dispensation. After these steps, all patients were referred for medical staff to authorize starting treatment. We performed the acceptance of pharmacist interventions accessing electronic medical records, new medical prescriptions, and by telephone follow-up. Hence, we divided patients into three different groups: 1) Drug Interaction Avoided (DIA), those with pharmacist interventions approved, 2) Drug Interaction Persisted (DIP), those pharmacist interventions not accepted for any reason; 3) no drug interaction (NDI).

c) Variables

The primary endpoint was SVR, defined as an undetectable viral load, three months after completion of DAAs therapy [7]. Among the secondary endpoints are: 1) number of DDIs (identified by drug or drug class); 2) severity of each DDIs according to HEP Drug Interactions –weak interaction, potential interaction and do not coadminister [8]; 3) number and types of pharmacist interventions classified as alter administration time, alternative medication, discontinuation, dosing adjustment, laboratory monitoring tests and monitoring for side effects.

d) Data sources/measurement

For the primary outcome, we used logistic regression to compare SVR rates between DIA, DIP and NDI groups. The results were collected from electronic hospital records and recorded on the database. To minimize the risk of bias, three authors (MSN, NLL, and GDRS) performed double-checking of all collected data presented in this study.

e) Study sample size

No sample size calculation was done before the conduction of this study. We recruited all patients from December 2015 to June 2017, who met the inclusion criteria. A post-doc analysis was conducted with G*Power [18]to estimate the achieved power of the primary outcome (association between SVR and groups of intervention by logistic regression), considering α=5% and observed effect size (OR), sample size and two-tailed regression model R^2.

f) Quantitative variables

The baseline characteristics include gender distribution, age, ethnic origin, DAAs regimen, treatment duration, HCV genotype, and presence of cirrhosis. We used frequency and percentage for categorical variables (total sample and for each group: DIA, DIP, NDI). We defined the continuous variables as mean and standard deviation.

g) Statistical methods

We compared the baseline characteristics between groups by the chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables. We used intention-to-treat (ITT) for missing data of SVR and obtained the odds ratio (OR) was obtained by a logistic regression model, including SVR as a dependent variable, groups (DIA, DIP and NDI) as independent variable and age, sex, genotype, and presence of cirrhosis as control variables. We

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considered the level of statistical significance to be 5%, and performed all analyses using STATA 13 (Stata Corp, Texas, USA).

V. Results

a) Participants

We included 1046 patients with chronic hepatitis C, with DAAs prescription. After Clinical Pharmacy guidance, patients were divided into groups DIA (n=273), DIP (n= 26), and NDI (n=747). In total, we excluded 74 patients (74/1046, 7.7%). Of these patients, ten had suspended treatment by adverse events, and 64 did not present the final HCV RNA-PCR test. No information was found about the death of patients on the electronic medical records.

b) Descriptive data

Overall, there was a ratio of 48.9% men, 51.0% women and mean age of 58.0 ± 11.42. A statistically significant difference of mean age was found between DIA and NDI groups. The ethnic majority consisted of Caucasians in all groups. The most common DAAs regimen was SOF + DCV + RBV (48.4%) and the overall treatment duration was 12 weeks (84.7%). We observed a higher frequency of genotype 1b (37.0%), followed by 1A (32.9%). Cirrhotic corresponded to 49.5% of overall patients, with statistically significant difference among the three groups. The baseline characteristics are available in table 1.

c) Outcome data

Our data showed a total of 299 patients (28.5%) identified with DDIs. In this group of patients, 273 had pharmacist intervention approved by the medical staff (DIA), and 26 were not approved (DIP). The total number of DDIs was 464, and 286 (61.6%) were identified only with DCV. For DCV interactions, identified drugs were composed of calcium channel blockers such as amlodipine, diltiazem and verapamil (n=85, 29.7%), followed by levothyroxine (n=59, 20.6%) and statins (n =46, 16.0%) (Table 2). The clinical pharmacists performed one hundred thirty-four interventions, such as alter administration time and 261 monitoring for side effects. Given the DDIs between DAAs and levothyroxine or warfarin, the medical staff accepted 54 interventions for laboratory monitoring tests (52.4%), and thirty-five alternative medication interventions (34.0%) because of contraindicated interactions between SOF, DCV, SMV, RBV, and drugs such as dipyrone (metamizole), anticonvulsants (phenobarbital, phenytoin, primidone, carbamazepine), amiodarone and dexamethasone. Paracetamol was recommendedfor medical staff to replace dipyrone. Drugs such as valproicacid, ethosuximide, lamotrigine and levetiracetam were recommended (after withdrawal) for patients with anticonvulsants prescription. Propafenone and prednisone were recommended to replace amiodarone and dexamethasone, respectively. Dosing adjustment (n=7, 6.7%) was requested for daclatasvir 90 mg and daclatasvir 30 mg (as a resultof CYP3A4 inducer – efavirenz and CYP3A4 inhibitor such as ritonavir, respectively). Moreover, dosing adjustments for amlodipine (10 mg to 5 mg/day), atorvastatin and simvastatin (both to 20 mg/day) because of potential interaction with DCV, were requested. In seven cases (n=7, 6.79%), discontinuation of drugs such as dipyrone, dexamethasone, orlistat, and colestyramine, was suggested for patients with no treatment indication. In the DIP group, 16 interventions for laboratory monitoring tests (61.5%), eight for the alternative medication (30.7%) –given the use of contraindicated drugs such as dipyrone, anticonvulsants, and dexamethasone, and two for DCV dosing adjustment were not approved. The acceptance rate of pharmacist interventions was 79.8% (Table 3). Our team identified three hundred thirteen drugs (68%) as potential interaction, 103 (23%) weak interaction, and 43 (9%) as "do not coadminister" (Figure 1). All identified DDIs by group (DIA and DIP) are available in Appendix A and B.

d) Main results

Intention-to-treat analysis (ITT) revealed an overall SVR rate of 80.1% (n=838/1046). In the DIA and DIP groups, SVR rates were 86.1% (n=235/273) and 57.7% (n=15/26), respectively. In the NDI group, 78.7% of patients (n=588/747) achieved SVR. The logistic regression compared SVR rates among the three groups.The results demonstrate that the DIA group had a greater probability of SVR compared to the NDI group (OR: 1.51; 95% CI 1.00 - 2.28; p=0.048). The DIP group had lower probability of SVR compared to DIA group (OR: 0.26; 95% CI 0.10 - 0.62; p=0.003) and NDI group (OR: 0.39; 95% CI 0.17 - 0.90; p=0.029) (Table 4). The post-doc analysis resulted in an estimated achieved power of 99%, considering α=5%, effect size=1.51, sample size=1046, and R^2=0.0361.

VI. Discussion

Our study shows the impact of pharmacist interventions related to DDIs on the clinical outcome of DAAs therapy in 1046 patients. Although we emphasize that our findings reveal an overall SVR of 80.1%, we present a larger sample of patients comparing to others real-life studies of Cheinquer et al. (n=219) [19], Ferreira et al. (n=296)[20]and AI444040[21](n=211). Cheinquer demonstrated the effectiveness of DAAs (SOF/DCV/SMV), 3D therapy (OBV/PTV/r + DSV) and SOF/ledipasvir (LDV), with or without RBV, and showed a higher SVR rate (>90%). Ferreira aimed to evaluate the effectiveness of (SOF/DCV/SMV/LDV/PEG-IFN) with or without RBV, while the clinical study AI444040assessed the effectiveness of SOF+DCV with or without RBV [21]. Both presented higher SVR rates

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(>90%). Despite this, samples of real-life studies were composed of 89 cirrhotic patients (42.7%) – Cheinquer,99 patients (38.8%) – Ferreira, and 30 patients (14.2%) – AI444040. In our analysis, 513 patients (49.5%) had cirrhosis. In Brazil, only patients with advanced liver disease (fibrosis and cirrhosis) or hepatitis B virus/HIV co-infected are given DAAs therapy [7]. Possibly, this is related to the results of the overall SVR rate in our findings. Nevertheless, in our data, patients have made use of concomitant drugs (n=299). Only Ottman, Townsend, Hashem, DiMondi, and Britt assessed the impact of DDIs on SVR in 300 patients [17]. This study evaluated patients on DAAs regimen (SOF, DCV, SMV, LDV, 3D, and elbasvir / grazoprevir), with or without RBV. In comparison to our results, a higher SVR rate was observed (95.6% vs, 80.1%). Ottman et al. did not found no statistically significant difference in SVR among patients who had at least one DDI compared with those who had not identified DDIs (94.8% vs, 95.8%; p=0.73). Also, 169 patients (56.3%) had advanced fibrosis or cirrhosis. However, the authors did not compare SVR rates in pharmacist intervention groups, as we presented, our logistic regression showed a higher probability of SVR in the DIAgroup, in comparison to DIP and NDI (Table 4). Possibly, this is related to the alternative medication (34.0%) in the DIA group, because interactions between DAAs and contraindicated drugs were solved. Likewise, there was a statistically significant difference of cirrhotic patients between the three groups. Regardless, our result highlights the role of the clinical pharmacist in the effectiveness of chronic hepatitis C treatment. This demonstrates that DDIs in DAAs therapy should not be neglected by the medical staff to avoid virological failure [9].

DDIs are also common in therapy with other DAAs. Maasoumy et al. demonstrated that 49% of patients were affected by DDIs with protease inhibitors (boceprevir and telaprevir), and management is required [6]. Other data suggest that the management ofDDIs can be performed by laboratory monitoring tests, dosing adjustment, alternative medication, or discontinuation, when necessary [6, 15, 17, 22]. Langness et al.[15] observed DDIs frequency with DAAs such as SOF/LDV, 3D, SMV/SOF, and SOF/RBV. Commonly recommended interventions for the management of each interaction were discontinuation (for contraindicated drugs, supplements, and herbal products), as well as monitoring for side effects. In our retrospective cohort study, the risk of self-medication and the use of supplements or herbal products (such as St. John's wort) were part of medication counseling for all patients. Therefore, we only consider the discontinuation intervention to those with DDIs in prescriptions. Besides Ottmanassess SVR, the author identified and quantified a total of 554 DDIs in 300 patients on DAAs therapy [17]. Ottman's study presented a greater focus on LDV/SOF and 3D. Only nine patients (3.0%) used SOF + DCV + RBV and of those, six had 11 DDIs identified (n=11/554 2.0%). Among the drug classes involved in DDIs, there are statins (n=87, 15.7%), calcium channel blockers (n= 63, 11.4%) and analgesics (n=48%, 8.6%). The most commons pharmacist interventions were dosing adjustment (29.6%), alternative medication (6.9%), and discontinuation (4.5%). Overall, 191 interventions were accepted (84.1%). We can compare our results of identified DDIs and the acceptance rate of pharmacist interventions. Our data present a higher frequency of laboratory monitoring tests (n=54, 52.4%) and alternative medication (n=35, 34%) approved interventions than dosing adjustment. This is explained by a higher proportion of patients using LDV/SOF or 3D scheme in Ottman's study. These DAAs act as inhibitors of various transporters (OATP1B1/3 OATP2B1, P-gp, BCRP) and different metabolic pathways (CYP3A4/5, UGT1A1, CYP2D6) in addition to inducing CYP2C19 [22].

Our study has some limitations. We instructed patients to do not start DAAs therapy until receive medical authorization (after medication counseling by Clinical Pharmacy), but we cannot guarantee that all patients followed this conduct. Probably, some have started treatment after medication counseling and dispensation. We advised patients about DDIs and several pharmacist interventions were performed by sending letters to the external medical staff. Possibly, some of them did not handed it to the medical staff and therefore, were included in DIP group. The clinical pharmacy staff advised all patients about the risks of self-medication and herbal product consumption. We told to avoid dipyrone during DAA therapy, because of the risk of interaction [8, 12]. Dipyrone is one of the most consumed over-the-counter drugs in Brazil [23], and we must consider the hypothesis that not everyone followed these advices.

Because of methodological limitations of a retrospective cohort study, we did not classify cirrhotic patients according to the Child-Pugh score. Probably, this would make it possible to understand SVR rates showed in our findings.

VII. Conclusion

Although the overall rate of SVR was lower than other real-life studies, our results indicate that the DIA group had a significant probability of SVR compared to DIP and NDI groups. Furthermore, this in DAA therapy are common and the medical staff should not neglect it. Pharmacist interventions may contribute to the effectiveness of DAAs therapy and makes it possible to avoid treatment failures caused by DDIs.

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Conflicts of interest: All authors have no conflict of interest to declare.

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Hatchability Dry Cysts and Morphological Effects of Newly Hatching Nauplii of *Artemia Salina* (Linnaeus, 1758) after Exposed to Tributyltin Chloride

By Najla Mohamed Abushaala, Syaizwan Zahmir Zulkifli, Ahmad Ismail & Abduo Fattah Mohamed Elfituri

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Keywords: *artemia cyst; acute-term mortality; ecotoxicology; hatching test; tributyltin chloride.*

GJMR-B Classification: *NLMC Code: QV 55*



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Hatchability Dry Cysts and Morphological Effects of Newly Hatching Nauplii of *Artemia Salina* (Linnaeus, 1758) after Exposed to Tributyltin Chloride

Najla Mohamed Abushaala [α], Syaizwan Zahmir Zulkifli [σ], Ahmad Ismail [ρ] & Abduo Fattah Mohamed Elfituri [ω]

Abstract- In previous studies focused on a nauplii stage of Artemia sp as a model to acute toxicity tests to detection of antifouling as an active agent against fouling marine organisms as Tributyltin Chloride (TBTCl). This research aims to investigate the toxicities of (TBTCl) on hatching dry cysts and morphological changes on newly nauplii of Artemia salina. The range of TBTCl concentration was selected (5, 10, 15, 20, 25, 50, 75, 100, 150, 200 ngl^{-1}). The results showes TBTCl significantly reduced hatching percentages of A. salina cysts from the (5 to 200 ngl^{-1}). The 200 ngl^{-1} TBTCl concentration showed no indication of hatching percentages among A. salina cysts. comparing with percentages in the control were 97%. The median effective concentration EC_{50} of TBTCl was (46.48 ngl^{-1}). The survivors nauplii were used to study the effect TBTCl on morphological malformation as total length and body width of newly nauplii. The higher rate of malformations of newly nauplii in 5 ngl^{-1} TBTCl concentration was 32.00 ± 4.62. Because in this concentration is a chance to newly nauplii survival to a longer period in toxic solution, which gives clearly deformities. While the lower deformities (%) were1.00 ± 0.00 at 75 ngl^{-1}. Because the chance to survival newly nauplii is very weak and it was difficult to observed the deformities clearly. As for the other concentration of TBTCl the deformities (%) was between this means. Conclusion, finding indicated that when increasing TBTCl concentration affected the hatching rate and TBTCl can killa embryo of A. salina cysts in higher concentrations, while in low concentrations can effect on morphological changes (total length and body width) when exposure dry cysts to seawater contaminated with TBTCl.

Keywords: artemia cyst; acute-term mortality; ecotoxicology; hatching test; tributyltin chloride.

I. Introduction

Recently there are many research about the acute toxicity of Tributyltin Chloride (TBTCl) on marine organisms. In this research studying the acute toxicity tests on *Artemia* sp. in previous studies focused on a nauplii of *Artemia* sp. This research aims to investigate the toxicities of (TBTCl) of the hatching stage of dry cysts and morphological changes of newly hatching nauplii of *Artemia salina*. In most scientific research widely used *Artemia* sp. as a model marine organism for ecotoxicity test, due to it is large geographical distribution. Despite it is popularity, the use of *Artemia* sp. in toxicity check is subjected to a wide discussion, at the global level, more often than not due to a number of criticisms about low sensitivity and lack of protocol standardization. (George-Ares et al., 2003; Mayorga et al., 2010; Leis et al., 2014; Libralato, 2014 and Rotini et al., 2015). Biological influences of TBTCl on *A. salina* may additionally furnish clues for of the accumulation mechanisms in coastal ecosystems as nicely as of the mode of action of TBT in these organisms. *A. salina* and different Artemia species have been used in the literature for the screening of acute toxicities of booster biocides (Bartolomé and Sánchez-Fortún, 2005; Koutsaftis and Aoyama, 2008 and Rotini et al., 2015). There are many advantages to use Artemia for example, adaptability to high temperature, adaptability to wide ranges of salinity, adaptability to varied nutrient resources, ease of culture, small body size and short life cycle (Nunes et al., 2006 and Koutsaftis and Aoyama, 2008). In addation, *Artemia* is low cost and can use it anywhere at any time.

Tributyltin chloriad is environmental hazards. The half of lives of tributyltin in the marine surroundings had been reported as nearly a number of days to weeks in water and frome one to ten years in sediments (Huang et al., 2004 and Al-Rashdi, 2011). In the previous studies toxicities of booster biocides have been reported on embryos of some marine organisms such as freshwater mussels, zebra mussels, blue mussels, sea urchins, oysters, and sea squirts (Bellas et al., 2007; Fent, 1996 and Wang et al., 2012). High concentrations of BTs have been detected in lower trophic animals such as caprellids. It appears that TBT accumulates specially in caprellids in the marine ecosystem, irrespective of the trophic level in the food

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chain, and that it may additionally establish breakpoint for disturbance in the herbal meals chain structure, therefore inflicting different organisms to accumulate TBT at increased concentrations due to their lower metabolic capacities to degrade TBT (Ohji et al., 2002a). in most of study about TBTCl was recognized as an environmental hazard and can effects on the early embryonic development of *Artemia salina*. They have proven toxic to embryos and larval stages of numerous aquatic organisms even at environmentally concentrations (Bryan and Gibbs, 1991). TBTCl a biocide added to antifouling paints to prevent the accumulation and attachment of algae and barnacles on the bottom of boats. TBTCl has become popular because it is effective, for a long time and causes environmental hazards to non-targeted estuarine organisms. This study aimed to investigate the effect toxicities of tributyltin chloride on hatchability cysts and study morphological changes of newly hatching nauplii of *A. salina*. And observe the morphological abnormalities of *A. salina* newly nauplii of completely hatched in each concentration of TBTCl toxicant and measurement the total length and width of the body (head width, abdominal width and tail width).

II. Materials and Methods

a) Hatching procedure and acute toxicity tests

The tributyltin chloride (TBTCl) used in toxicity tests was kindly provided by Sigma-Aldrich, USA (purity 96%). Stock solutions of TBTCl were prepared by diluting with artificial seawater up to 35‰ salinity. The range of concentration TBTCl was selected as (5, 10, 15, 20, 25, 50, 75, 100, 150, 200 ngl^{-1}). The experiments were performed in 50 ml test tube within tube racks that were submerged in water up to the midpoints of the tubes. Constant aeration, illumination (1000 Lux), and temperature (28 ± 1 °C) were maintained and the replicate number was three in the experiments each replicate 100 cysts of *A. salina* cysts. For each test group, added 40 ml from the test solution of different concentration TBTCl to test tubes, and then a 24 hours hatching period was initiated. After the hatching period, the number of newly hatched nauplii, viable hatched, cysts, and malformation newly hatched nauplii were counted. Hatching percentages (HPs) of cysts were determined by counting the number of completely hatched of nauplii. Hatching failure (found by subtracting the number of completely hatched nauplii from total group size). After that account hatchability (%), Deformity (%) and viable hatchability (%) by using the following formulae (Revathi and Munuswamy, 2010).

Hatchability (%) = 100* (no. of hatched larvae) / (no. of total cyst in test)

Deformity (%) = 100*(no. of deformed larvae)/ (no. of hatched larvae)

Viable hatchability (%) = 100*(no. of viable hatchability larvae in test)/ (no. Total cyst in test).

b) Median effective concentration (EC_{50})

The data on the hatchability inhibitor % of cysts was used in the estimation a 50% effective concentration (EC_{50}) in different concentration of TBTCl. The effective concentration EC_{50} values were determined by using probit analysis in XL TEST-Pro (version 2014.5.03). And each end point was calculated by using the following formulae (Shimasaki et al., 2003).

c) Morphological abnormalities

The morphological abnormalities of *A. salina* newly hatched nauplii of completely hatched in each concentration exposed of TBTCl toxicant were observed under magnification (10x) using a Leica M 205 stereomicroscopy attached to a camera with an aid of software (Easy-Grab; Noldus Information Technology) and the total length and width of the body (head width, abdominal width and tail width) have been measured (Alyuruk and Cavas, 2013).

III. Results and Discussion

Effects of Tributyltin Chloride on Cysts Hatchability in Artemia salina

1. Hatching Percentages (%)

Hatchability of the exposed *A. salina* cysts to different concentrations of TBTCl observed in this study is presented in *Figure 1*. The hatching percentages were shown to be affected by TBTCl concentrations. TBTCl significantly reduced hatching percentages of *A. salina* cysts at the various concentrations by using the following formulae (Revathi and Munuswamy, 2010). Hatchability (%) = 100* (no. of hatched larvae) / (no. of total cyst in test). The hatching percentages in the control were 97%, which is within the reported value of the manufacturer (minimum of 90% hatchability). Among these completely hatched cysts, 76% were active and 21% were viable hatched (completely hatched, but still not active). The remaining 3% cysts were found hatched after hatching period was prolonged for more than 24 to 48hr. From the results observed a significantly decrease hatching percentages from the 5 to 200 ngl^{-1} and was TBTCl had varying effects on the hatching percentages of *A. salina* cysts (Figure 1). In 200 ngl^{-1} showed completle hatching inhibition percentages of *A. salina* cysts. The cysts exposed to TBTCl within 24hr were unable to hatch even the hatching period was prolonged until 48hr. This result confermed TBTCl can kill a embryo and inhibit hatchability of *A. salina* cysts.

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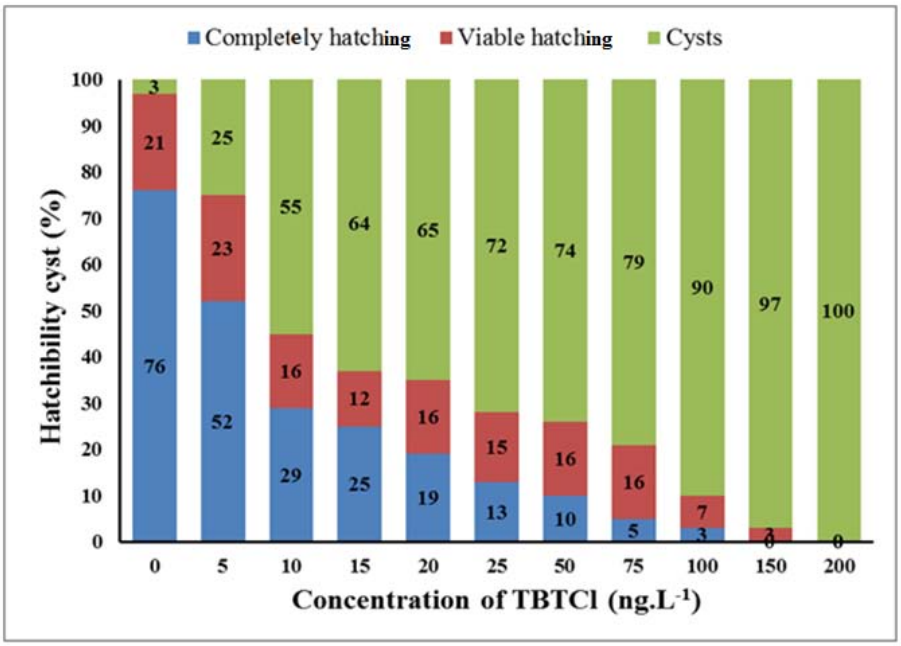


Figure 1: The cysts hatchability percentage of *A. salina* after exposure for 24-h in different TBTCl concentrations [Completely hatching = active nauplii, Viable hatching = nauplii in membrane embryo, Cysts = unable to hatch]

A. salina cysts are a barrier to withstand external environmental condition. Disruption of these activities by certain concentration of TBTCl bioaccumulated into the cysts may cause death to the dormant embryo, and this finding is relative agreement with the results of this research have shown that the toxicity of TBTCl can impact the hatching process of *A. salina* cysts. This result is supported through Brix et al., (2006) studied estimated the median high-quality concentrations (EC_{50}s) for metallic salts, suggesting that the hatching end point for *A. franciscana* is the most touchy examined to date for steel salts in saline environments and same in sensitivity with the most touchy tested to date for Cu. But in present finding *A. salina* cysts are more sensitive to TBTCl at lower concentration 5 ngl^{-1} it was 25% cysts comparative with the control samples 3% cysts, and that mean TBTCl can inhibits hatching process and can kills dormant embryo in the low concentration and also when increasing the TBTCl concentration. Revathi and Munuswamy, (2010) investigated the effects of TBT on the embryonic development, and hatching success of eggs uncovered to TBT in the freshwater prawn brooder *Macrobrachium rosenbergii*, and observed TBT at 3.12 ppm, delayed the embryonic development and significantly reduced the hatchability of eggs as well. two on the different hand, the treated businesses showed impaired embryonic development with reduced body growth. Thus, TBT has appreciably retarded the embryonic improvement in the freshwater prawn *M. rosenbergii*. These studies clearly demonstrated the possible effects of toxicants particularly TBTCl on unhatched eggs or cysts of crustaceans, including *A. salina* was more sensitive to TBTCl at 5 ngl^{-1}, and the possible reason that TBT is more toxic to *A. salina* because the body size is small and it is life cycle is very short *Figure 2* shows effect of different concentrations of TBTCl on the performances hatching of *A. salina* cysts percentages. *Figure 2 (a)* shows the completely hatching (%) that mean the newly hatching nauplii is active and healthy as shown in control. Several nauplii exposed to 10, 25 and 50 ngl^{-1} wereviable hatching (%) that mean the newly hatching nauplii completely hatching, but still not activeand in the embryonic membrane (*Figure 2 (b), (c) and (d)),* while *A. salina* cysts exposed to 75 ngl^{-1} TBTCl concentration was unable to completely break the cyst wall (*Figure 2 (e)*), while the *A. salina* cysts in the 100 ngl^{-1} unable to hatching (*Figure 2 (f)*). This sequence of effects relatively showing the severity of TBTCl as its concentration increase in the aquatic environment.

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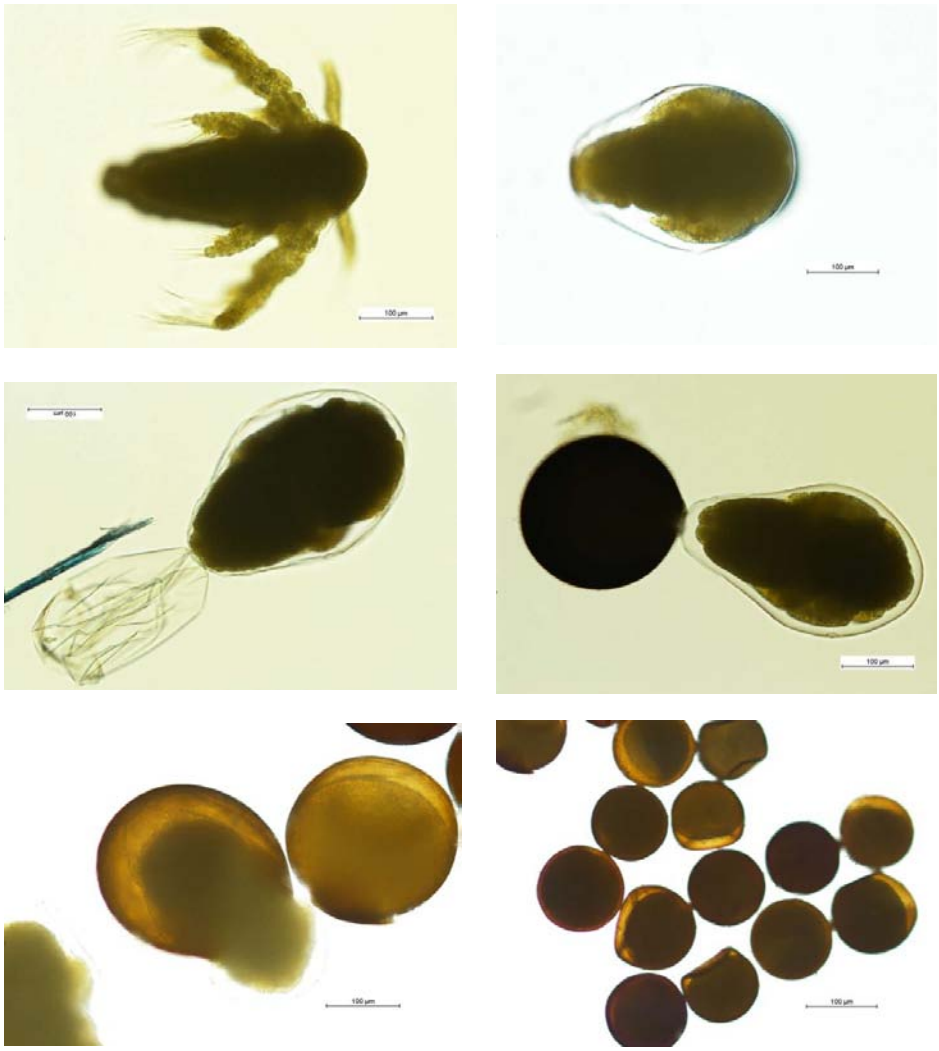


Figure 2: Effects of Increasing TBTCl Concentration on the Performances Hatching Percentages (%) HPs of *A. salina* Cysts. [Remark: (a) control = Completely hatching, b) 10, c) 25, and d) 50 ngl^{-1} = Viable hatching, e) 75 ngl^{-1} = unable to complete break the cysts wall, and f) 100 ngl^{-1} = unable to hatch *A. salina* cyst. [Bar: 100 μm].

2. Median Effective Concentration (EC_{50}).

The median effective concentration EC_{50} of TBTCl as shown in (*Figure 3*), at different concentration of TBTCl the *A. salina* nauplii completely hatching after 24hr was (EC_{50} 46.48 ngl^{-1}), this is mean the TBTCl impacted the process of hatchability *A. salina* cysts and significantly reduced the hatchability cysts when increasing the concentration of TBTCl. Since there is a confined research on the inhibitory effects of the hatchabilty share cysts of *A. salina* it was once two examine EC_{50} of TBTCl with one of a kind toxicants such as metals have been pronounced on the hatchability percentage of cysts for instance (Caldwell et al., 2003) studied *A. salina*, have been observed to inhibit hatching success of *A. salina* cysts in dose. A higher sensitivity was once discovered in the 24 and 72hr publicity EC_{50} for 24hr was once 2.14 and 72hr was 0.023 μg ml^{-1}. This result is an settlement with (Brix et al., 2006) studied estimated the EC_{50} of metallic salts are suggesting that the hatching endpoint for *A. franciscana* is the most sensitive examined to metals in marine environments. Meanwhile, Alyürük and Çavaş, (2013) mentioned their investigation related to the toxicities of diuron to the hatching stage of *A. salina*, their results showed that diuron should be a attainable hatching enzyme inhibitor and used to be substantially lowered the hatching proportion of *A. salina* cysts and prevented the hatching of cysts. Rotini et al., 2015 said in their learn about *Artemia* sp hatching assay is a touchy choice device to acute toxicity take a look at and the hatching test resulted extra touchy than acute mortality tests. The outcomes show the reliability and excessive sensitivity of this hatching assay on a short time and guide it is a useful application of first tier risk assessment techniques in the marine environment.

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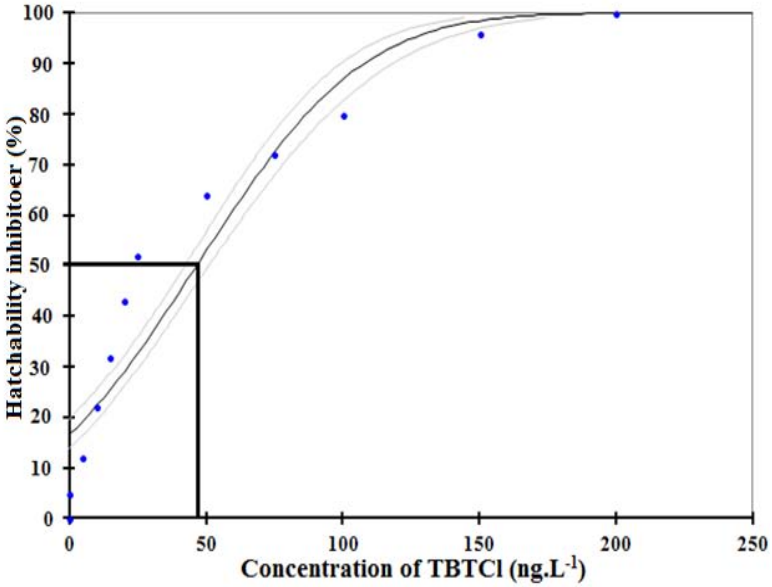


Figure 3: Median Effective Concentration 50% (EC_{50} = 46.48 ngl^{-1}) between TBTCl Concentration and Hatchability Inhibition (%) of *A. salina* Cysts

3. Morphological Effects of *A. salina* Newly Hatching Nauplii after the Exposed the Cysts to Different Concentration of TBTCl

Analysis of the morphological deformities (%) of the means *A. salina* newly hatching nauplii after the exposed the cysts to different concentration of TBTCl as shown in *Table 1*. The higher rate of deformities of newly hatching nauplii at 5 ngl^{-1} TBTCl concentration was 32.00 ± 4.62. Because in this concentration is a chance to newly nauplii stay a longer period survival, which gives greater opportunity to appear changes in shape and deformities. While the lower morphological deformities (%) were1.00 ± 0.00 at concentration 75 ngl^{-1}. Because the chance to survival newly nauplii is very weak and continued growth and development to the body is slowly and it is difficult to note the deformities clearly. As for the other concentration of TBTCl the deformities (%) was between this means. That means the TBTCl different concentration can impact the morphological changes in newly hatching nauplii when exposure the dry cysts to artificial sea water contaminated with TBTCl.

Table 1: Morphological Deformities (%) of Means and SE of Newly Hatching Nauplii *A. salina* Affected by Different Concentration of TBTCl [N: numper of cysts]

TBTCl (ngl^{-1})	N	Deformity (%)
0	300	0.00 ± 0.00^{a}
5	300	32.00 ± 4.62^{c}
10	300	16.33 ± 3.76^{b}
15	300	$9.00 \pm 0.58^{a, b}$
20	300	$9.67 \pm 1.46^{a, b}$
25	300	$6.33 \pm 1.76^{a, b}$
50	300	2.33 ± 0.33^{a}
75	300	1.00 ± 0.00^{a}

Because in this concentration is a chance to newly nauplii stay a longer period survival, which gives greater opportunity to appear changes in shape and deformities. While the lower morphological deformities (%) were1.00 ± 0.00 at concentration 75 ngl^{-1}. Because the chance to survival newly nauplii is very weak and continued growth and development to the body is slowly and it is difficult to note the deformities clearly. As for the other concentration of TBTCl the deformities (%) was between this means. That means the TBTCl different concentration can impact the morphological changes in newly hatching nauplii when exposure the dry cysts to

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artificial sea water contaminated with TBTCl in the low concentrations.

The morphological deformities such as total length in the newly hatching nauplii as can be seen in the increase concentration of TBTCl, the total length of newly hatching nauplii *A. salina* will significantly decrease in general total length in newly hatching nauplii were represented in *Table 2*. This table is shown means of total length newly hatching nauplii shows the control group was (350.9 ± 49.6) μm, but the means for total lengths in different concentration of TBTCl 5,10, 15, 20, 25, 50, 75 ngl^{-1} were (284.6 ± 51.6), (266.8 ± 54.6), (282.2 ± 59.3), (294.9 ± 40.6), (288.8 ± 45.7), (274.8 ± 39.7) and (269.8 ± 54.6) μm, respectively. According to mean and standard error the lowest total length was (266.8 ± 54.6) μm at 10 ngl^{-1} TBTCl concentration compared with the control group.

Table 2: Total Length of Newly Hatched Nauplii After 24hr Exposed to Different Concentration of TBTCl [N: numper of nauplii]

TBTCl (ngl^{-1})	N	Mean ± SEM (μm)	Minimum (μm)	Maximum (μm)
0	35	350.9 ± 49.6^{d}	314.3	451.7
5	35	284.6 ± 51.6^{c}	221.6	377.8
10	35	266.8 ± 54.6^{a,b,c}	202.6	364.2
15	35	282.2 ± 59.3^{a,b}	212.5	398.9
20	35	294.9 ± 40.6^{c}	223.8	389.9
25	35	288.8 ± 45.7^{b,c}	216.5	352.1
50	35	274.8 ± 39.7^{b,c}	244.8	393.3
75	35	269.8 ± 54.6^{a}	201.6	304.2

The morphological changes such as total length and width of body in the newly hatching nauplii as can be seen in *Figures 4, 5, 6 and 7*. In general the regression analysis (*r*) shown the high regression values and this demonstrates a strong inverse relationship between morphological measurements and increase TBTCl concentration. The head width is more affected compared to the total length r = 53 %. And the head is more caricatures and more sensitive to increasing of TBTCl r was 95%. While the abdomen and tail width of body shown moderately affected r = 89 % and decrease when the increasing concentration of TBTCl. In present study need to mention there are not enough studies about effects of TBTCl on the morphological changes in newly hatching nauplii so will be compare these findings with similar studies about nauplii exposed to different types of toxins. For example, Abushaala et al., (2015a) study effect of TBTCl on nauplii stage of *A, salina* and reported in their results the TBTCl had effect the morphology changes of nauplii *A. salina*. On the other hand, (Rao et al., 2007) studies toxicity of Organophosphates on morphology changes in nauplii *A. salina* and significant morphological alteration were noticed in nauplii. In under study Abushaala et al., (2015b) studied effect of Diorun on nauplii stage of *A. salina* their results shown the Diorun had effect the morphology changes in the nauplii stage of *A. salina*. Also Anderson, (2009) his find out about confirmed the impact of alcohol proportion on the improvement rate of *A. salina* nauplii.

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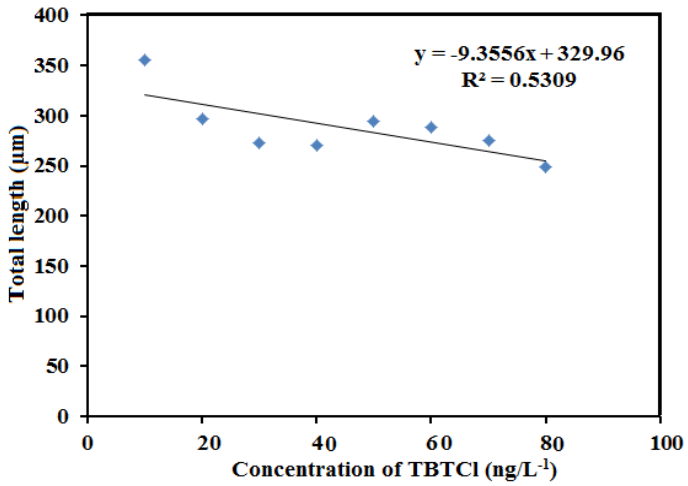


Figure 4: Morophological of Total Length *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

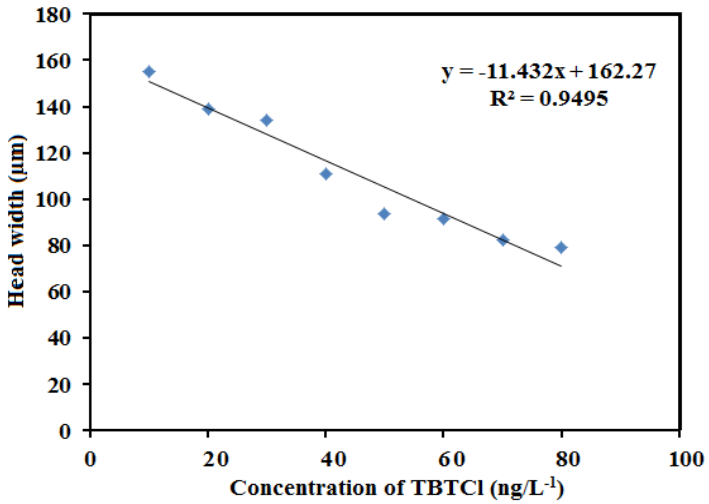


Figure 5: Morphological of Head Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

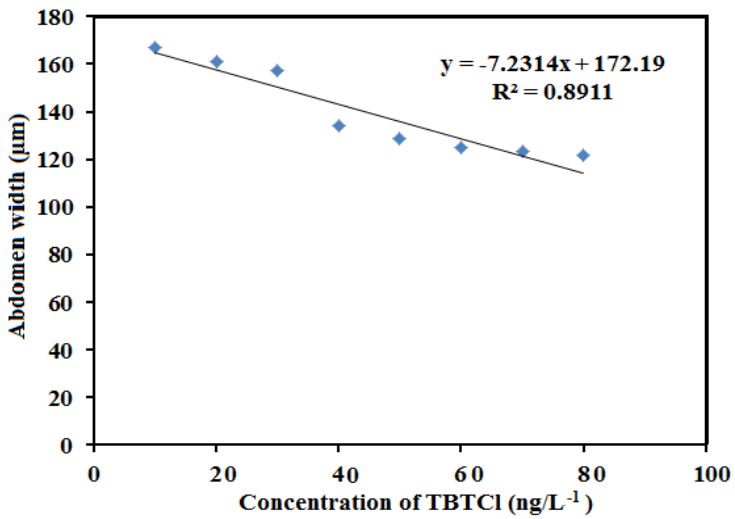


Figure 6: Morphological of Abdomen Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

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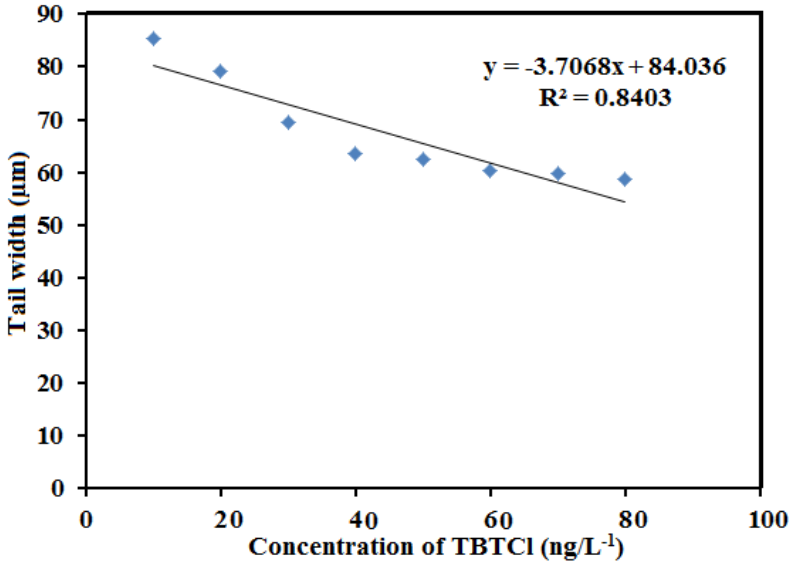


Figure 7: Morphological of Tail Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

IV. Conclusion

In this study increased TBTCl concentration in solution could significantly decreased the hatchability percentage of *A. salina* cysts and prevented the hatching of larvae. And the EC_{50} value of TBTCl was once recognized as 46.48 ngl^{-1} after 24hr exposure. Early nauplii of *A. salina* is sensitive to TBTCl contamination to reflect hatchablity cysts and early life stage effects of toxicant. In addation, the TBTCl effect on morphological abnormalitiy active newly nauplii *A. salina*. And significant morphological differences were observed in all nauplii exposed to the different concentration of TBTCl are used in this research. These results indicate that in this system TBTCl, it is proven environmentally toxic substances. In general, these results indicated that when increasing TBTCl concentration affected the total body length and the body width of *A. salina* newly hatched nauplii. In spite this result indicated that the system TBTCl is acutely toxic.

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Assessment of Antibacterial Activity and Minimal Inhibitory Concentration of Leaf Extract of Morinda Citrifolia Against Enterococcus Feacalis- An Invitro Study

By Venkata Teja Kavalipurapu, Apoorva Vasundhara Kaligotla & Gummuluri Sriram

Abstract- *Background:* When root canal infections are concerned, the causative factor is primarily the microbial inhabitation, which leads to the spread of infection. The literature evidence supports the aspect that frequent usage of antibiotics induces resistance and threatens the effectiveness of the treatment. So, the current issue that is faced globally is the development of resistant microbial species. To combat this interest, towards the usage of newer herbal horizons is increased.

Materials and Methods: The antibacterial effect of Ethanolic Leaf Extract of Morinda Citrifolia was investigated against Enterococcus Faecalis (E. Faecalis). Agar well diffusion and broth dilution methods were used to determine the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Results: The MIC of Ethanolic Extract of Morinda Citrifolia extract was found to be 250 μg/ml, and the MBC was 400 μg/ml

Keywords: *bacteria, biofilm, endodontics, morinda citrifolia leaves, root canal.*

GJMR-B Classification: *NLMC Code: QV 744*



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Assessment of Antibacterial Activity and Minimal Inhibitory Concentration of Leaf Extract of Morinda Citrifolia Against Enterococcus Feacalis An Invitro Study

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Abstract- *Background:* When root canal infections are concerned, the causative factor is primarily the microbial inhabitation, which leads to the spread of infection. The literature evidence supports the aspect that frequent usage of antibiotics induces resistance and threatens the effectiveness of the treatment. So, the current issue that is faced globally is the development of resistant microbial species. To combat this interest, towards the usage of newer herbal horizons is increased.

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Conclusion: Ethanolic leaf extract showed better antimicrobial activity on E. Faecalis.

Keywords: bacteria, biofilm, endodontics, morinda citrifolia leaves, root canal.

I. Introduction

Endodontics is a field where complex root canal anatomy, dealt in a simplified way. Most of the treatment and therapeutic success attributed to the complete removal of tissues and contents from root canal space, including the dentinal and microbial debris (1),(2). When root canal infections are concerned, the causative factor primarily is microbial inhabitation, leading to the spread of infection into periapical space, causing apical periodontitis(3). It's a known fact that root canal infections cannot be attributed to a single species or a single microbe. The pathogenic resistant microbial colony is attributed to the formation of a complex biofilm(4),(5). So, the major hurdle in therapeutic endodontics is the complete removal of bacterial biofilm(5).

So, from the previous discussion, it's understood that microbial inhabitancies are primary causation factors for root canal infections. Thus, therapeutic options and advancements tend to concentrate more on this aspect. Although the advancing front is towards the discovery of antimicrobial agents in eradicating the bacterial biofilm, it's more neglected that the threat of antimicrobial resistance is also increasing. So, the current issue that is faced globally is the development of resistant microbial species(6). The literature evidence supports the aspect that, frequent usage of antibiotics induces resistance and threatens the effectiveness of the treatment (7),(8).

The evolving trend in new therapeutics is concentrating on a wide range of herbal products and natural plant extracts. The evidence also supports that these products exhibit a broad range of antimicrobial properties and therapeutic benefit(9),(10). So, many researchers and clinicians have shifted their interest in exploring the natural plant extracts and assessing their therapeutic effects. Although there is a wide range of natural products and plant extracts widely used for various medicinal purposes, the well-documented usage and benefits in endodontics are seen majorly with Morinda Citrifolia(11).

A study by Murray et al.,(12) stated that, the antimicrobial efficacy of Morinda Citrifolia juice was similar to 6% sodium hypochlorite, which is considered as a gold standard root canal disinfectant in endodontics. The currently available evidence is strongly in favor of sodium hypochlorite as the main endodontic irrigant(13).

The concentration of hypochlorite used and the contact time of hypochlorite with root canal walls deals with its effectiveness in reduction of microbes (14), especially E. Faecalis, which is known to be a resistant species responsible for the reinfections(15). But, the sad truth is that the choice of irrigant concentration and usage cannot be standardized in the clinical scenario as it is possible in an experimental scenario. So, in a clinical scenario, various studies compiled and showed evidence of developing a resistant E. Faecalis in failed primary root canal treatments(16),(17). Hypochlorite at lower concentrations might lead to the development of resistant strains (14).

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So, this was the primary concern for the authors of the present study. We state that "Herbal products although might not replace primary root canal irrigant, but might be an adjunctive therapeutic option and a lot of research and therapeutic potential, still lack and to be translated in a clinical scenario". So, our research majorly concentrates on assessing various plant extracts. Our previous research has focused on assessing the Ethanolic Fruit Extract of Morinda and proved to be beneficial against E. Faecalis(9). So, the present study formulated was to assess a step ahead to evaluate the beneficial antimicrobial effect of Ethanolic Leaf Extract of Morinda Citrifolia. The null hypothesis stated was, there was no significant antibacterial effect of ethanolic leaf extract of Morinda Citrifolia against E. Faecalis on using agar well diffusion and broth dilution methods.

II. Materials and Methods

The study was approved by the Institutional Ethical Committee. Before the start of the research, the sun-dried powder of Morinda Citrifolia was collected. The extract preparation was similar to our previous study(9), where we used the dried fruit and seeds powder against E.Faecalis. In this present study, we concentrated mainly on the effect of dried leaf powder. The dried leaf powder was initially subjected to 1000ml of ethanol using Soxhlet extractor for 72, not exceeding the boiling point of the solvent. After which, the extract was filtered and vacuum dried at 45 degree Celsius. The obtained extract was refrigerated until use.

The strains used for testing were gram-positive E. Faecalis bacterial strains ATCC 29212. The bacterial strains were cultured in Luria broth agar (Himedia, Mumbai) and incubated at 37-degree celsius for 24 hours and maintained on nutrient agar slants at 4-degree celsius. The sterile spreader was used for the inoculation of these organisms across the media. 10mg/ml of sample was provided for analysis.

Determination of Minimum Bactericidal Concentration:

The microorganisms were inoculated into molten Luria broth agar and poured into Petri dishes and solidified. Wells of uniform diameter were then prepared on the solidified agar. The discs were then impregnated with experimental test solution at different concentrations of 40, 60, 80, and 100μl, respectively. 10mg/ml of tetracycline was used as a positive control, and the solvent without plant extract was used as a negative control. Plates were incubated for 24 hours at 37-degree celsius, and the development of the inhibitory zone around the wells was measured in diameter and recorded.

MBC value was determined by subculturing the test dilution on a freshly prepared nutrient agar media. The plates were incubated further for 18-42 hours at 37-degree celsius. The highest dilution yielded no visible turbidity on nutrient agar and taken as MBC.

Determination of Minimum Inhibitory Concentration:

The MIC of the test solutions was determined using broth dilution methods using CLSI 2012 standard protocol (18).

The cultures were then incubated and subsequently, serially diluted to reach a density of 2×104 cells per ml. Cell counting was done using a hemocytometer. Luria broth (Himedia, Mumbai) was prepared and sterilized at 121-degree celsius, 15lbs for 15 minutes.

Two milliliters of Luria broth was dispensed into the tubes, and 100μL of cell culture was inoculated in it. Different concentrations of ethanolic leaf extracts of 7.5, 15.625, 31.25, 62.5, 125, 250, 500, and 1000μg respectively were added into the tubes. Positive and negative controls were similar to agar well diffusion. Growth control was run parallel with every experiment. All the experimental tubes were incubated for 48 hours in anaerobic jars. After completion of the incubation period, the optical density was measured at 600nm. Each experiment was carried out in a triplicate set. The lowest possible concentration before the color change was considered as MIC. The percentage of bacterial inhibition was computed by an equation, as mentioned in our previous study(9).

III. Statistical Analysis

Data were analyzed using SPSS version 11. Multiple comparisons were made using one way ANOVA followed by LSD test for post hoc analysis. Statistical significance was considered for $p<0.05$.

IV. Results

Table 1 depicts the MBC, and Table 2 illustrates the MIC values. Each value represented as a mean ± standard deviation. When results were compared, the statistically significant difference was noted as compared to the negative control.

V. Discussion

From the results of the present study, it can be concluded that the ethanolic extract of morinda citrifolia leaf also seemed to have an efficient antibacterial activity on assessing using agar well diffusion and broth dilution methods. None of the previous literature on this specific aspect can be compared, as much of the literature is concentrated on the pathogenic medical microorganisms. When the results of the present study were compared with our previous study results, they showed inferior values on both MIC and MBC evaluation. So, it can be assessed that fruit extract seemed to have better antimicrobial properties as compared to leaf extract on ethanolic extraction.

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Previous literature has reported the potent antimicrobial activity in the organic solvent as compared to water as an extracting compound, which indicated that active compounds resulting in antibacterial activity are more soluble in organic solvents (19). When the antimicrobial property of the herbal or natural plant products is evaluated critically, the enhanced effect cannot be attributed to a single compound or agent. It's a combined action of various bioactive compounds such as alkaloids, flavonoids, and other significant compounds promoting its activity (20).

Previous literature on morinda citrifolia has shown enormous evidence based data on its antimicrobial, antifungal, antioxidant, and anticancer properties (21), (22), (23). As discussed previously, the combined action of bioactive compounds in the plant extract of morinda citrifolia, which led to its enhanced antibacterial activity. The null hypothesis of the present study rejected, and results proved that leaf extract of morinda citrifolia possessed antimicrobial property on assessment.

When a limitation of the present study is considered, it's a first invitro study simulated to assess a single pathogen. But, when root canal infections are considered, it's usually polymicrobial inhabitations (24). So, the effectiveness of the agent tested by this in-vitro study is not possible. So, better future studies should concentrate on combined agents on multiple endodontic pathogens to prove their effectiveness. In our perspective, there is still a long way for the natural plant extracts to be considered effective in clinical endodontics.

VI. Conclusion

Ethanolic leaf extract of morinda citrifolia seemed to possess antimicrobial properties against E. Faecalis and can be considered as an antimicrobial agent to treat root canal infections.

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Career | Credibility | Financial

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Acknowledgments

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

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j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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Author details

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Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

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Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

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22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

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The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

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- Submitting a manuscript with pages out of sequence.
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- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

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- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
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Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

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Figures and tables:

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

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- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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