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Inhibition of the Human Hepatitis C Virus by Dibenzyl Trisulfide from *Petiveria alliacea* L (Guinea Hen Weed)

Henry I. C. Lowe[1,2,3*], Ngeh J. Toyang[1,2], Sanjit Roy[1], Charah T. Watson[3] and Joseph L. Bryant[2]

[1]Educational and Scientific LLC, Baltimore, MD, USA.
[2]Institute of Human Virology, University of Maryland, School of Medicine, Baltimore, MD, USA.
[3]Bio-Tech R&D Institute, Kingston, Jamaica.

Authors' contributions

This work was carried out in collaboration between all authors. Authors HICL and NJT designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors NJT and CTW carried out the studies. Authors SR and JLB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The anti cancer and anti diabetic properties of the extract from the *P. alliacea* a naturally occuring plant found in Jamaica, have been described previously but its role against hepatitis C virus (HCV) infection is completely unknown. The aim of the study was to evaluate the anti HCV activities of the *P. alliacea* extract and its isolates dibenzyl disulfide (DDS) and dibenzyl trisulfide (DTS).
Methodology: Luciferase and cell cytotoxic activities were measured using the extracts of

**Corresponding author: E-mail: henrylowe@cwjamaica.com;*

P. alliacea, DDS and DTS.
Results: The crude extract of *P. alliacea* and DTS inhibited the HCV expression while DDS was inactive. The EC_{50} of the crude ethyl acetate fraction was 18.0 µg/ml while DTS was 5.69 µg/ml and the reference compound rIFN α-2b was 0.57 IU.
Conclusion: Our results suggest that the extract of *P. alliacea* is a promising antiviral agent against HCV. To the best of our knowledge, this is the first report about the inhibition of HCV expression mediated by *P. alliacea and* DTS. Further studies are required to determine the mechanism of action of DTS against HCV.

Keywords: Hepatitis C virus; Petiveria alliacea; Guinea hen weed; luciferase; Dibenzyl trisulfide.

1. INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem and emerges as both an endemic and a pandemic live threating disease. HCV is known to be the major cause of hepatocellular carcinoma which is the 3rd leading causes of cancer death in the world [1]. The HCV virus is a single stranded RNA virus which was first discovered in 1989 [2]. There are at least six genotypes of HCV and the distribution is region specific with some genotypes more prevalent in some regions than in others. The infection is transmitted mainly through blood transfusion and amongst children, vertical transmission is one of the main modes of transmission of the disease. Approximately, 170-185 million peoples are infected with the HCV and 3-4 million peoples are newly infected every year with the virus [3-5] and the US has approximately 2.7 million people infected with HCV [6]. An estimated 23000-46000 children are infected with HCV in USA, increasing the projected $10.6 billion medical cost of HCV treatment by at least $200 million for the next decade [7]. The consequences of untreated HCV infection is dangerous asprolonged infection with HCV is now considered a major cause of liver chirosis, fibrosis and hepatocellular carcinoma resulting in about 700,000 deaths annually from the infection [3,5,8]. HCV infection is also related to the development of other complications like lymphoma, diabetes, artherosclerosis among other illnesses [9,4,10-12].

For treatment of HCV infection, interferons have been used for a long time as the mainstay of treatment [13]. The most well known therapies are Pegylated-Interferon + Ribavirin with or without a protease inhibitor such as Boceprevir/Telaprevir. These treatments have a cure rate or rate of sustained virologic response of 66-80%. This treatment regimen is very costly and not all patients do respond in the same way. Some patients show intolerance and or contraindications to these common therapies. People infected with HCV and who end up developing carcinoma may often require organ transplantation [14]. Natural sources remain a promosing avenue for the discovery and development of new medicines for treating patients due to the vast diversity of natural products and their chemistry.

Petiveria alliacea L. is a medicinal plant called Guinea Hen Weed in Jamaica. The plant possesses anti spasmodic, anti rheumatic, anti inflammatory [15], nociception [16], anti hypoglycemic and anti tumor properties [17,18]. The bioactive compound isolated from the plant was characterized as Dibenzyl trisulfide (DTS), which showed potent anti cancer and anti viral activity. The DTS has been found to inhibit MAPK pathway kinases like RSK [18]. DTS specifically inhibit the C-terminal kinase domain of RSK1 and showed broad spectrum anti proliferative activity on several cancer cell lines including prostate, breast, pancreatic, and lung [18]. Recent studies showed that the crude plant extract and its bioactive compound DTS play an important anti HIV-1 role by inhibiting the HIV-1 reverse transcriptase activity [19].

Although the crude plant extract and its derivative had been shown to possess potent activity against many cancer cells [17,18,20] its role against HCV infection has not yet known. In this study, the anti HCV potential of the plant *P. alliacea* and its bioactive compound is described.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Luciferase assay kit was purchased from promega, USA. Human Hepatitis C (HCV) virus reporter was obtained from [21]. Interferon α was from Schering-Plough (New Jersey, USA), CytoTox-1 reagent (Promega, USA); Dibenzyl

disulfide (DDS), (Sigma Aldrich, USA); Dibenzyl trisulfide (DTS) (International 66 Laboratory, USA).

2.2 Plant Collection and Extraction

The *Petiveria alliacea* plant was collected from Jamaica, West Indies. The extraction of bioactive compound from the plant has been described previously [20].

2.3 Cell Culture and Growth Condition

Human hepatic carcinoma cell lines (Huh 7.5) was obtained from ATCC. Cells were grown and maintained in Dulbeccos modified essential media (DMEM) with L-Glutamine (invitrogen, USA), supplemented with 10% FBS, 1% penicillin-streptomycin, 1% Non-essential amino acids; (Invitrogen, USA) in a humidified incubator with 5% CO_2, at 37℃.

2.4 Luciferase Activity Assay

The cell line Huh 7.5 expressing recombinant HCV- Renella luciferase with JFH1 virus used in this study has been described previously [21]. Briefly, $1x10^4$ cells were seeded in each well of a 96 well plate. The test agents were diluted with DMEM with 5% FBS in a volume of 50 ul equal to the cell culture volume. The test compounds were used in different concentrations with six serial dilution (0-100 ug/ml) in triplicate. The highest concentrations of the agents used were; rIFN α-2b 10 u/ml and 100 µg/ml for GHW-E DTS and DDS respectively. After 72 hr treatment, cells were lysed for measurement of luciferase activity using Renella Luciferase Assay system, according to the manufacturers instructions (Promega, USA). The number of viable cells in each well were determined by Cyto Tox-1 reagent (Promega, USA) All data were analyzed and graphed using Excel.

3. RESULTS

The pharmacologic properties of the extract and compounds are shown in Table 1. Treatment of the cells with the compounds resulted the following activities, EC_{50}: For IFN α 0.57 IU, GHW-E 18 µg/ml, DTS 5.69 µg/ml, DDS > 100 µg/ml. The IC_{50} were: IFN α > 10 IU, GHW-E 73.8 µg/ml, DTS 6.53 µg/ml, DDS >100 µg/ml. The selective indexes were: IFN α > 17.50 GHW-E4.1, DTS 1.15, DDS >100.

HCV-luc expression data are shown in Fig. 1. Luciferase activity showed that interferon α drastically suppressed the HCV expression concentration dependently. Cell viability was not affected with interferon α treatment, Fig. A.

Table 1. Anti HCV activity of the *P. alliacea* and DTS

Extract/ compound (SI)	EC_{50}	IC_{50}	Selectivity index
rIFN α-2b	0.57	>10.0	> 17.50
GHW-E	18.00	73.80	4.10
DTS	5.69	6.53	1.15
DDS	>100	>100	>100

SI= IC_{50}/EC_{50}. EC_{50} and IC_{50} values were determined by variable slope non-linear regression analysis of the plotted data using GraphPad Prism software. GHW-E: Ethyl acetate extract of P. alliacea

GHW-E also showed reduced activity of HCV – Luc and with 100 ug/ml there was substantial suppression. Cell viability was reduced at this concentration Fig. B. DTS showed HCV-luc suppressive capability which was very drastic at 100 ug/ml. Cell viability was also very low at this concentration Fig. C.

4. DISCUSSION

Development of new antiviral drugs is essential to overcome problems like cost and ineffectiveness using the current therapies. The effect of chemotherapy from natural sources on HCV is very poorly known. To increase knowledge in this area, reporter gene assays with HCV fused in Renella luciferase system was conducted. The substantial reduction of luciferase activity in cells treated with the bioactive compounds compared to untreated cells indicated that *P. alliacea* posseses antiviral activity. To ascertain the experimental reliability Interferon α-2b was used as a positive control. Interferon α 2b has been used for a long time against HCV infection [22]. Since a previous report [19] showed that DTS has potent cytotoxic effect, the influences of the cytotoxic activity of the drugs on luciferase activity were determined. From the cytotoxic activity assay it is clear that interferon α exerted almost no cytotoxic activity whereas the samples of GHW tested showed cytotoxicity at higher doses. The selectivity index (SI) and EC_{50} of GHW-E and DTS suggests that GHW-E and DTS might be a potential remedy for HCV. Our previous study showed that *P. alliacea* extracts have anti HIV activity by inhibiting HIV-1 reverse transcriptase [19]. Although the detail molecular action of the extract on HCV infection is not known at this time, the results from the current study suggest that similar mechanism

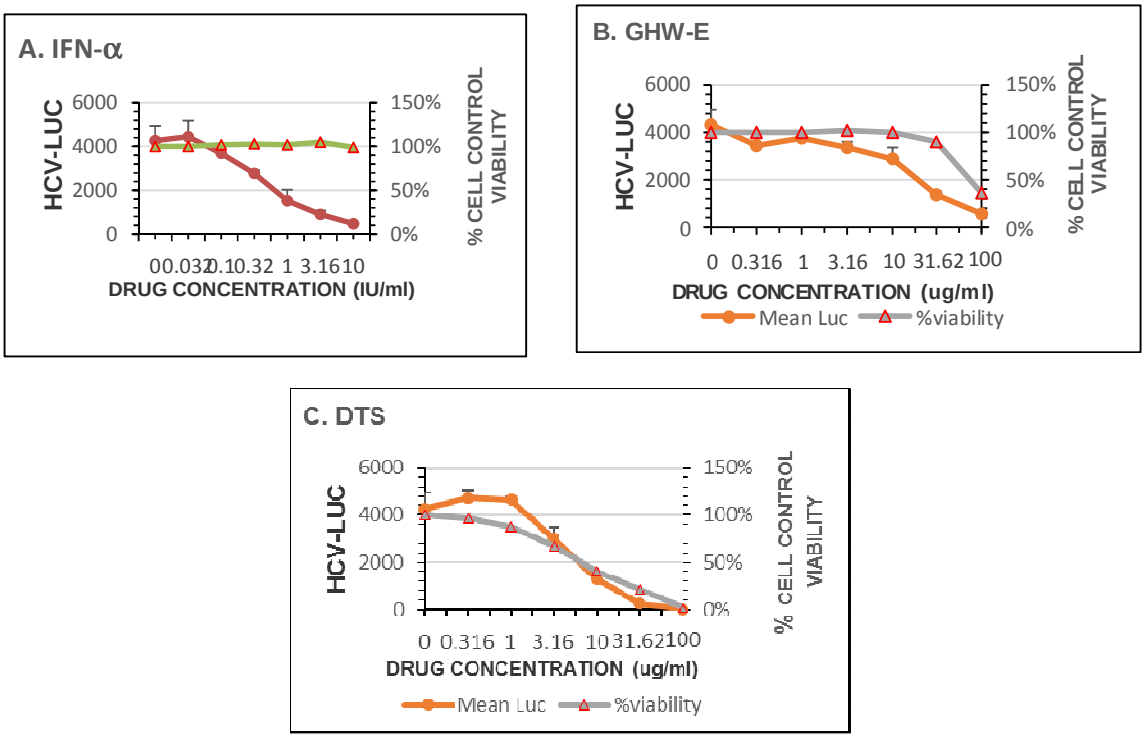

Fig. 1. Inhibition of Hepatitis C virus infection mediated by *P. alliacea* extract and its major metabolite DTS

A. Human rIFNa was used as a positive control. B. Bioactive compound (GHW-E) isolated from P. alliacea. C. DTS compound. The virus infection was measured by Renilla luciferase activity and cell viability was measured by cytotoxicity assay. Activity expressed as LUC unit; HCV-LUC, cell viability expressed as % of control. The error bar indicated the mean variation of the experimental error

may be involved in inhibting HCV. The results also suggest that by inhibiting HCV infection, the extract may inhibit the onset and progression of hepatocellular carcinoma; an end stage HCV disease of the liver. The results from previous studies [19] and present study suggest that GHW-E and DTS may be very useful for the treatment of patients infected with both HIV-1 and HCV. Substantial evidence have shown that HIV-1 and HCV infection activate map kinase pathway [23,24]; which is required for virus particle production and infection. Since DTS possess anti map kinase potential [18] is further evidence that strongly supports the potential application of *P. alliacea* and DTS against viral infections where more effective therapies are lacking. Again, further in vivo studies are needed to validate the therapeutic application of this valuable medicinal plant.

5. CONCLUSION

The role of the antiviral activity of naturally occuring plant compounds is of great interest from both the economic and safe uses point of view. There is growing evidence of the anticancer properties of *P. alliacea* extract and DTS [25,17,18] and their anti viral activities are being revealed [19]. *P. alliacea* supplements are available in Jamaica, West Indies as a therapy for several conditions including cancer and diabetes. The findings reported herein provide further evidence on the possible broad spectrum bioactivity properties of *P. alliacea*. To the best of our knowledge, this is the first report about the inhibition of HCV by *P. alliacea* extract and purified compound DTS. The major limitation in preclinical drug discovery for HCV is the lack of a small animal in vivo model which makes the validation of in vitro results difficult. Further studies are needed to the therapeutic uses of the plant extract against chronic hepatitis C virus infection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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