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Research Article

Effect of the combination of *Silybrum marianum* and *Lycopodium clavatum* on some biological markers for monitoring chronic hepatitis B virus patients

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Abstract

Viral hepatitis B is a disease for which no curative treatment is available to date. The objective of this study is to evaluate the effect of *Silybrum marianum* and *Lycopodium clavatum* preparations on the biological parameters of viral hepatitis B follow-up.

Material and methods: This was a longitudinal study of a period of 5 months. The study population consisted of consenting chronic hepatitis B patients. A questionnaire was used to collect sociodemographic information and medical history of the patients. One Step Rapid Test HBV-2210305 and Biolabo kits were used to test for serological parameters and transaminases. The viral load was determined using the real-time PCR technique. The decoctions were made from the leaves and rhizomes of *Sylibum marianum* associated with the floral juice of *Lycopodium clavatum*. The evaluation of the effect of the decoction was done by comparing the values of parameters between the beginning and after 3 months of treatments.

Results: Seven out of nine patients were chronically infected with the hepatitis B virus in active phase. After 90 days of treatment, we noted the persistence of AgHbs in all patients but a disappearance of AgHbe in 75% of patients. Mean transaminase activity and viral load decreased significantly after treatment (P=0.045).

Conclusion: The preparation of a decoction of *Silybrum marianum* and *Lycopodium clavatum* leads to normalization of Alanine Amino transferase activities (AAT), loss of HBe antigen and decrease of viral load after three months of treatment.

Keywords: Chronic hepatitis B, *Silybrum marianum, Lycopodium clavatum*, decoctions, transaminase activity and viral load.

INTRODUCTION

Viral hepatitis B remains a global public health concern.[1] In 2019, the World Health Organization (WHO) estimated that 296 million people were living with chronic viral hepatitis B, among whom there were 820,000 deaths related mainly to cirrhosis and hepatocellular carcinoma.[1] Africa represents an area of high endemicity with an estimated prevalence of 8% in West Africa, 5-7% in southern, eastern and central Africa.[2] This high morbidity is mainly due to low vaccination coverage, low awareness, screening and low standard of living.[3]

To fight viral hepatitis B, the WHO recommends a treatment based on nucleotide analogues of the reverse transcriptase associated with pegylated interferon. This treatment is not curative, but it does reduce the viral load and prevents the patient from developing the complications of liver cirrhosis and hepatocellular carcinoma (HCC).[4] In the African context, access to this treatment remains limited due to its high cost of treatment. According to the WHO report in 2020, more than 102 million chronic carriers are not on antiviral therapy.[5,6] Faced with this problem, patients are turning to traditional health care providers for low-cost natural plant-based care.

In the light of the incidence and prevalence of viral hepatitis B, which is still high in Black Africa in particular, researchers and herbalists are constantly exploring molecules that can bring a definitive cure to patients. For example, a study conducted in Mali revealed that some traditional recipes resulted in 6.80% HBsAg negativity without any effect on creatinine levels.[7] Another study conducted in Benin by Mouzouvi et al in 2014 shows that the combination of Combretum micranthum and Cochlospermum tinctorium extracts lead to a normalization of the ALT level with a seroconversion of the HBsAg of 4.17% in patients.[8] Other authors notably Nencini et al (2006), Gordon et al (2006) and Samader et al (2013) have demonstrated that other plants notably Cassia occidentalis and Lycopodium clavatum are endowed with immunostimulant and repressive properties of HeLa cancer cells proliferation respectively.[9,10,11]

Sylibrum marianum is another hepato-protective plant whose therapeutic effects are no longer to be demonstrated. Indeed, this plant possesses bioactive substances able to induce the regeneration of hepatocytes and inhibit the formation of leukotrienes.[12] Other authors have demonstrated that silymarin contained in S. marinum increases by 10 times the secretion of interferon-alpha (IFN-a) by lymphocytes and attenuates tumor necrosis factor responsible for the

normalization of serum aminotransferase levels while preventing graft reinfection after orthotopic liver transplantation in patients with chronic decompensated hepatitis C virus infection. [13,14]

Opposite to the different plants with hepato-protective potential mentioned above, we did not find any work evaluating their effects in the management of patients suffering from viral hepatitis B in Cameroon despite the increase in morbidity and mortality related to hepatocellular carcinoma estimated at more than 10,000 deaths per year.[15] At the same time, we did not find any work related to the evaluation of the combination of Silybrum marianum and Lycopodium clavatum on HBe antigenemia, viral load and liver fibrosis grade in patients with hepatitis B in replicative phase, although these are the first line markers of therapeutic efficacy evaluation.[16] In view of this observation, we decided to carry out this work with the aim of evaluating the effect of the association of Silybrum marianum and Lycopodium clavatum in the management of chronic viral hepatitis B.

General objective: Evaluate the effect of traditional recipe of these three plants on the viral load, the renal and hematological function of chronic patients carriers of Hepatitis B virus (HBV)

Specific objectives

1. Study the state of carriage of the hepatitis B virus of patients at the beginning of treatment

2. Determine the effect of traditionnal treatment on the viral load of chronic patient carriers of HBV associated to HBs antigen charge after three (03) months of treatment

3. Evaluate de recipe effect on the hepatocellular function of these patients after 3 months

MATERIAL AND METHODS

Study population and sampling

The study population was composed of chronic hepatitis B virus carriers confirmed in hospital and received at the Centre for the Promotion of Medicinal Plants of Noun for treatment. Indeed, when these patients were received by the phyto therapist, the interest of the study was explained to them. For those who consented by signing the informed consent form, the sociodemographic data and the therapeutic history of the patients were recorded using a survey form. To ensure the viral serological status of the patients, 5ml of whole blood were collected in EDTA tubes and dried from all patients for analysis of biological markers of disease monitoring such as: HBsAg, HBsAb, HBeAg, HBeCA, HBeCAc, AST, ALT and viral load.

Ethno-pharmacological survey and description of therapeutic regimens

The ethno pharmacological survey allowed us to identify the two plants Silybum marianum and Lycopoduim clavatum used by the phytotherapist in the treatment of chronic hepatitis B in West Cameroon. The leaves and rhizomes of these two species; and seeds of Lycopoduim clavatum are the main organs most used. This drug was prepared by decoction from powder following a protocol similar to that Benmarce et al in 2018 and Aloui et al in 2016.[17,18] This preparation was administered for 90 days with 1 glass morning, noon, and night in patients without abdominal distension. Regarding patients expressing signs of cirrhosis, the treatment is made on the basis of floral juice combined with the decoction obtained previously. The treatment is based on the floral juice combined with the decoction obtained previously. The dosage used in this case is three tablespoons morning, noon and evening per day for the first 30 days and for the last 60 days the dosage is that of the decocted powder of the two plants.

Sampling collection

During the treatment, controls were being realized after three (03) months (J90) in other to evaluate the effectiveness of recipes. Followed up files of these different variables were elaborated in other to collect the followed up data. Blood samples were recorded in dry and EDTA tube adapted on vacutaner before the treatment and after the three months in other to evaluate their effect in the laboratory.[1]

Study design

We conducted a longitudinal study which took place during from January 2020 to May 2020. A questionnaire was used to collect datas on the HBV infection status and the sickness history on 25 participants. Among these participants, 5 received the conventional treatment and were excluded from the study. The 20 participants remaining seropositive to antibodies directed against the capsid antigen IgG of Hepatitis B virus (anti-HBc IgG) and surface antigen (HBs Ag) carriers were included. The screening of the serological markers(anti-HBc IgG, HBs Ag, HBe Ag, anti-HBe and anti-HBs) , dosage of hepatoprotective markers (ALT and the determination of the fibroses grade) and quantification of viral DNA were done to these participants at the inclusion. They were submitted to the traditional treatment. After three (3) months of followed up five (5) participants were lost of view, six (6) were excluded for poor therapeutic observance. Blood sample was recorded from the nine (9) remaining participants for a new re-evaluation of the recipes effect on the sero-convertion of the HBs and HBe antigen, viral load, ALT activity and the fibroses of patients (Fig 1).

Study of the viral carriage state before the treatment at T0 days

Before starting the patients on the therapeutic regimens described above, the HBs and HBe antigens and their respective antibodies were detected by the ONE STEP RAPID TEST HBV-2210305 Kit (5 parameters). The optical density of HBs was measured by ELISA kits (DIA.PRO, Milan, Italy) at the Bethanie laboratory.

The hepatic transaminases (ALT and AST) were quantified from the serum obtained after centrifugation at 3000 rpm for 5 minutes. The assays were performed according to the enzymatic method on a Cobas®6000 following the recommendations of Tietz et *al*.[17]

Subsequently, the viral carriage profiles obtained were described based on the European Association for the Study of the Liver protocol.[16]

Effect of treatment on viral load

Viral load was determined from plasma samples from patients. Briefly, 5 mL of blood was centrifuged at 4000rpm for 10 minutes. The resulting plasmavwas stored at -20°C until the DNA was extracted using the Abbott® protocol. The amplifications targeting the S region of the HBV genome were performed using the m2000 RealTime™ (Abbott®).

Effect of treatment on the sero-conversion of HBs and HBe antigens after 90 days of treatment (T 90 days).

After 90 days of treatment, blood samples were again taken from patients to reanalyze viral biomarkers by the same kits used above. The impact of treatment on these markers was assessed by comparing the values obtained at the beginning of treatment with those expressed by the patients after 90 days.

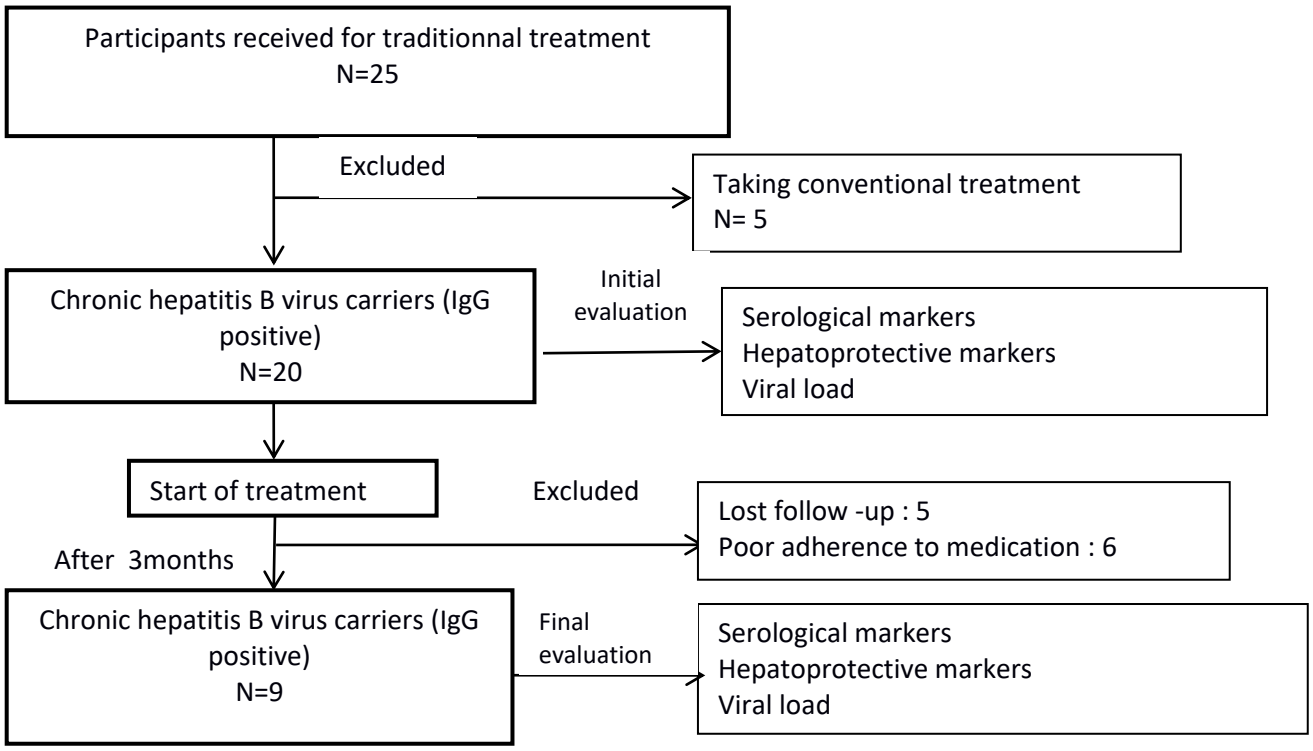

Figure 1: Flow diagram showing the study population

Effect of treatment on hepatocellular function of chronic carriers (ALAT and FIB-4)

This evaluation was done in order to assess the effect of the preparations used on the hepatocellular function. In this part we first proceeded to the determination of the ALT at the initiation of the treatment and after 90 days in all the patients included. Subsequently, the platelet concentration was determined using Mindray 5150 flow cytometry and the aspartate amino transferase activity was determined after 90 days of treatment. These obtained values associated with the patient's age allowed indirect calculation of the fibrosis grade by the FIB-4 method (EASL, 2017) whose formula is as follows:

FIB = Age (year) x AST (IU/L) /Blood platelets (10^9/L) x (ALT (IU/L)) $^{1/2}$

FIB-4< 1.45 patient with 90% chance of not developing fibrosis.

FIB-4> 3.25 patient with extreme fibrosis or cirrhosis in 65%.

Statistical analysis

Data were analyzed with SPSS 20.0 software. Continuous variables were tested for normal distribution and expressed as mean ± standard deviation or median. Mean ALT values and HBs antigen optical density at baseline were compared with posttreatment values using the paired-sample Student t-test. P value <0.05 was considered significant.

RESULTS AND DISCUSSION

Results

In this study, 9 of 25 patients agreed to participate to this study for a participation rate of 36%. Among the 09 chronic carrier patients, 7 (77.78%) were male and 2 (22.22%) were female. The sex ratio was 3.5 in favor of men. Single people were the most represented in this work with a rate of 55.56%. The majority of our patients (55.55%) were between 35 and 45 years old, followed by the 15 to 25 years old with a rate of 33.33%. These results are recorded in Table 1 below.

Table I: Sociodemographic characteristics of chronic hepatitis B patients

Variables	Frequency n(%)
Sex	
Female	2 (22,22)
Male	7 (77,78)
Matrimonial Status	
Single	5 (55,56)
Maried	4 (45,44)
Years	
15 – 25	3 (33,33)
25 – 35	5 (55,55)
35 – 45	1 (11,12)

Pathological states of patients obtained after detection of viral markers and ALT assay at the beginning of treatment are summarized in Table 2 below. This result reveals a heterogeneity of viral B pathological processes with a predominance of cases of chronic hepatitis B in immunological clearance phase followed by chronic active hepatitis B.

Table 2: Pre-treatment viral carriage pathological status in patients at baseline to treatment (EASL, 2017).

Pre-exposure viral carriage status	**Effectifs**
Chronic hepatitis B in the immunological clearance phase, HBeAg (+), ALT>40UI/L et 0,22x 10^5UI/ml	4 (44,44%)
Chronic active hepatitis B HBeAg (-), ALT>40UI/L et 0,101x 10^5UI/ml	3 (33,33%)
HBeAg chronic hepatitis B (-) ,Normal ALT and undetectable DNA	2 (22,23%)

Table 3 below highlights the effect of the treatment on the patients' disease states. It appears from this table that the intake of decoctions made with Silybum marianum and Lycopoduim clavatum leads to a loss of HBe antigen of 75% in patients initially in immunological clearance phase accompanied by a normalization of the ALT level of 71.42% (5/7) in carriers at risk of hepatic fibrosis after 90 days.

Table 3: Effect of treatment on disease states in chronic carriers

	Chronic hepatitis B in the immunological clearance phase HBeAg (+), ALT>40UI/L CV : 0,22x 10^5UI/ml	**Chronic to active hepatitis B, HBeAg (-), ALT>40UI/L CV: 0,101x 10^5UI/ml**	**HBeAg chronic hepatitis B (-), Normal ALT and undetectable DNA**
Effectif at t=0	4	3	2
Effectif at t= 90 days	1 (3/4)	1(2/3)	2
HBeAg seroconversion rate and ALT normalization	75% (3/4)	66,67% (2/3)	

Table 4 below shows the main changes in ALT and HBsAg optical density from initiation of treatment to 90 days. A significant decrease in ALT level (P=0.045) from 98.25 to 38.00 IU/L can be seen compared to the HBsAg optical density which remains almost stationary.

Table 4: Variation in ALT level and HBsAg OD from t0 to t90 days of treatment

Variable	**T0**	**T90 days**	Mean of the difference	**IC à 95%**	**Student's statistical test**	**P-value**
ALT (IU/L)	98,25	38,00	60.250± 84.280	-10.210 à -130.710	2.022	0.045
DO HBsAg	16,41	15,98	0.42250± 4.280	-3.53230 à 4.37730	0.253	0.808

Study of the effect of the treatment on the average viral load compared to the average titer of HBsAg obtained in active patients shows in table 5 below that decoctions made with Silybum marianum and Lycopoduim clavatum lead to a decrease of 90.65% of the initial viral load ranging from 1605 to 1500 IU/ml This observation would justify the significant decrease in alanine amino transferase activity observed in Table 4. HBsAg would not be the biological marker of choice in the evaluation of therapeutic efficacy due to the poor correlation between ALT and HBsAg absorbance (r=0.20 P=0.62).

Table 5: Change in viral load in a control compared to the mean HBsAg optical density in the sample.

Measuring time	Viral load (UI/mL)	**Measuring time**	Variation in mean optical density of HBsAg
T0	16050	**T0**	16,4100
T90	1500	**T90**	15,9875
Effect (DNA repression rate)	90,65%	**Effect (percentage of HBsAg loss)**	2,82%

Study of the effect of the treatment on the hepatocellular function by the calculation of the FIB-4 recorded in table 6 reveals that the extracts Silybum marianum and Lycopoduim clavatum prove to be protective for the hepatocellular function. Of the 77.73% (7/9) chronic carriers in active phase at risk of developing fibrosis or cirrhosis estimated at 65% at the beginning of treatment, only 14.28% (1/7) of patients had the same risk of developing this complication after 90 days.

Table 6: Characteristic of the hepatic fibrosis grade of patients after 90 days of treatment

FIB-4 values at t 0	Effectifs	FIB-4 values at t=90 days	Effectif
<1,45	2 (22,22)	<1,45	8 (88,88)
> 3,25	7(77,73)	> 3,25	1(14,28)

DISCUSSION

The present study aimed to evaluate the effect of medicinal preparations made from leaves and floral parts of Silybum marianum and Lycopoduim clavatum on some biological markers of follow-up of chronic hepatitis B virus carrier patients. Our sample is characterized by a male predominance admitted for treatment of chronic hepatitis B in immunological and active clearance phase. These profiles are in agreement with the World Program against viral hepatitis B and C which stipulates that only this target group can receive antivirals in order to reduce the risk of development of cirrhosis from a chronic state which would be more pronounced in men than in women.[18,19] Most of these patients are between 26 and 30 years old with an average age of 28.88 ± 6.91 years. This is not surprising, as this is the period when sexual activity is more intense and sometimes uncontrolled.[20]

Taking decoctions and floral juices made from Silybum marianum and Lycopoduim clavatum had no effect on HBsAg, yet this preparation caused a disappearance of HBeAg in 75% of chronic carriers in the immune clearance phase after 90 days of treatment. These results are contrary to those of Mouzoui et al (2014) and Djiguiba (2005); who had shown a disappearance of HBsAg in 4.17% and 3.70% of patients respectively without any effect on HBeAg.[21,22] The difference in results observed could be explained by the difference in the composition of secondary metabolites of the plants used and consequently the difference in pharmacological effects.[23] Indeed, S. marinum and L. clavatum contain mainly silymarin and lycopodin with antiviral properties.[24] On the other hand, C. micranthum and C. tinctorium are rather highly concentrated in tannins and caffeine which are powerful anticancer and diuretic agents facilitating a rapid purification of HBsAg.[9,25]

In addition to this seroconversion in HBe antigen, we observed at the same time a significant normal decrease of ALT activity in 71.42% (5/7) of chronic carriers in active phase who became inactive. These observations are not surprising insofar as, independently of the plant considered, in addition to their antiviral properties, they lead to a normalization of alanine aminotransferase activity.[12,13] These therapeutic effects are also thought to be associated with the silymarin and lycopodine contained in these two plants respectively, which are able to increase the secretion of interferon-alpha (IFN-a) by lymphocytes by 10-fold and consequently attenuate the tumor necrosis factor responsible for the decrease in alanine aminotransferase.[25,26,27]

The decrease in the initial mean viral load of 90.65% highlighted in these chronic carriers who became inactive is consistent with the loss of HBe antigen. Indeed, in addition to the pharmacological properties assigned to the above drugs, it is clear that they possess, in addition to metabolites with antiviral activity, enzymatic analogues of pepsin and metallo protease, which confer them the capacity to interfere with viral replication and also the translation of structural proteins, preventing the production of protein "e" and, by ricochet, of protein "S".[28,9,27]

The biological effects associated with these two plants have contributed to improve the hepatocellular function through the decrease of the risk of hepatic cirrhosis. Indeed, the risk of cirrhosis decreased from 65% to 10% in chronic carriers in active phase who became inactive after 90 days. These observations can be attributed to the anti-inflammatory properties of the flavonoids contained in these medical preparations. Indeed, the loss of the HBe antigen suppresses the cell-mediated immune response and inactivates the inflammatory process responsible for hepatocyte lysis and thus a normalization of ALT.[29,30,31]

CONCLUSION

It appears from our study that the preparations made with Silybrum marianum and Lycopodium clavatum improve the health status of chronically active patients by causing a decrease in viral load, a loss of HBe antigen and consequently the normalization of ALT activity after 90 days of treatment. In view of these effects, these preparations may constitute an alternative to the treatment of chronic viral hepatitis B in developing countries like ours.

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Conflict of interest: We confirm that there are no known conflicts of interest associated with this publication.

REFERENCES

1. World Health Organization. Hepatitis B World Health Organization,2020; Department of Communicable Diseases Surveillance Response. WHO/CDS/CRS/LYO/2015.2.

2. Kurbanov F, Tanaka Y, Fujiwara K, Sugauchi F, Mbanya D, Zekeng L, Ndembi N, Ngansop C, Kaptue L, Miura T, Ido E, Hayami M, Ichimura H, Mizokami M "A new subtype (subgenotype) Ac (A3) of hepatitis B virus and recombination between genotypes A and E in Cameroon" Journal of general Virology, 2017; 86:2047-2056. https://doi.org/10.1099/vir.0.80922-0

3. Bigna J, Marie A, Serra L, Angeladine M, Steve R, Elodie T, Jean J, "Seroprevalence of hepatitis B virus infection in Cameroon: a systematic review and meta-analysis "British Medical Journa, 2016; 6:101-136. https://pubmed.ncbi.nlm.nih.gov/28667212/

4. Raffetti E, Fattovich G, Donato F,"Incidence of hepatocellular carcinoma in untreated subjects with chronic hepatitis B: a systematic review and meta-analysis" J Hepatol ,2016; 36:1239-51. https://onlinelibrary.wiley.com/doi/pdf/10.1111/liv.13142 https://doi.org/10.1111/liv.13142

5. Akpemi, A.M, (). Cytotoxicity Activity and Phytochemical Screening of Cochlospermum tinctorium Perr Ex A. RichRhizome. Journal of Applied Pharmaceutical Science , 2012; 2(7):155159.https://app.dimensions.ai/details/publication/pub.1073687359?and_facet_journal=jour.113798

6. Zoulim F, Locarnini S, (2009)" Hepatitis B virus resistance to nucleos(t)ide analogues" Gastroenterology , 2009; 137:1593-608. https://doi.org/10.1053/j.gastro.2009.08.063

7. Djiguiba M. Evaluation de trois recettes dans le traitement traditionnel de l'hépatite B au Mali.Thèsedepharmacie, 2005; 94p.

8. Mouzouvi R, Djègo J, Sehonou J, Lalèyè A, Priuli F, Bigot A" Effet de l'association Combretum micranthum G. Don (Combretaceae) et Cochlospermum tinctorium A. Rich. (Cochlospermaceae) dans la prise en charge de l'hépatite virale B. Série Pharm Journal of African Traditional Medicine, 2014; 17(1):10-14. https://www.google.com/search?q

9. Nencini C, Giorgi G, Micheli Protective effect of silymarin on oxidative stress in rat brain" Phytomedicine,2006; 2:1-40. https://pubmed.ncbi.nlm.nih.gov/16638633/

10. Gordon A, Hobbs D, Bowden D, (2006)" Effects of Silybum marianum on serum hepatitis C virus RNA, alanine aminotransferaselevels and well-being in patients with chronic hepatitis C" Journal of Gastroenterol Hepatol, 2006; 21:275-280. https://pubmed.ncbi.nlm.nih.gov/16460486/ https://doi.org/10.1111/j.1440-1746.2006.04138.x

11. Samadder A, Das S, Das J, Paul A, Boujedaini N, KhudaBukhsh AR, "The potentized homeopathic drug, Lycopodium clavatum (5C and 15C) has anti-cancer effect on Hela cells in vitro" Journal of acupuncture and meridian studies, 2013; 6(4):180-187. https://pubmed.ncbi.nlm.nih.gov/23972240/ https://doi.org/10.1016/j.jams.2013.04.004

12. Dehmlow C, Erhard J, de Groot H "Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin" Hepatology,1996; 23(4): 54-749. https://pubmed.ncbi.nlm.nih.gov/8666328/ https://doi.org/10.1002/hep.510230415

13. Da S, Barros P, Silva G, Landi M "Hepatoprotective effect of Lycopodium clavatum 30 CH on experimental model of paracetamol-induced liver damage in rats" Homeopathy, 2015; 104:29-35. https://pubmed.ncbi.nlm.nih.gov/25576269/ https://doi.org/10.1016/j.homp.2014.05.005

14. Freedman N, Curto T, Morishima C, Seeff L, Goodman Z "Silymarin use and liver disease progression in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis trial" Aliment Pharmacol Ther, 2011; 33:127-137. https://www.google.com/search?q https://doi.org/10.1111/j.1365-2036.2010.04503.x

15. Neumann U, Biermer M, Eurich D, Neuhaus P, Berg T,"Successful prevention of hepatitis C virus (HCV) liver graft reinfection by silibinin mono-therapy" Journal of Hepatol 2010; 52(6):951-2. https://www.google.com/search?q https://doi.org/10.1016/j.jhep.2010.02.002

16. Hamid K, Zahra S, Sayed Mehdi H,Taghi G, Vahid S, (2011)"Effects of silybum marianum on patients with chronic hepatitis" Journal of Reseach in Medical Sciences , 2011; 16(3): 287-90. https://pubmed.ncbi.nlm.nih.gov/22091246/ 17. Marie A, Serra L, Asangbeh, Steve R, Noumegni N, Elodie T"Seroprevalence of hepatitis B virus infection in Cameroon: a systematic review and meta-analysis" Journal of Infectious diseases, 2016. https://bmjopen.bmj.com/content/7/6/e015298 https://doi.org/10.1136/bmjopen-2016-015298

18. European Association for the Study of the Liver(EASL) " Clinical practice guidelines on the management of hepatitis B virus infection" J Hepatol, 2017; 64(6):1388-402. https://doi.org/10.1016/j.jhep.2016.11.004

19. Benmarce Y, Assas N, Chaalal A, La silymarine (chardon-marie), de puissantes propriétés anticancéreuses et hépatoprotectrices. Mémoire de master en Toxicologie et Santé; 2016. P1-36. https://www.google.com/search?q=Benmarce

20. Alaoui SE . Valorisation de deux plantes marocaines Melia azedarach et Silybum marianum. Thèse de doctorat en Chimie et environnement ; 2016. P.106-107. http://www.abhatoo.net.ma/maalama-textuelle/developpement

21. Tietz . Clinical Guide to Laboratory Tests. 1996 ; 3rd ed. AACC

22. Liaw YF, Lok A " Follow-up and indications for liver biopsy in HBe Ag-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review" Journal Hepatol, 2012; 57:196-202. https://doi.org/10.1016/j.jhep.2011.11.030

23. Bréchot C "Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and newparadigms"Gastroenterolog, 2004; 127:S5661.https://www.sciencedirect.com/science/article/abs/pii/S0016508504015951. https://doi.org/10.1053/j.gastro.2004.09.016

24. Fomulu J, Morfaw F, Torimiro J "Prevalence, correlates and pattern of Hepatitis B among antenatal clinic attenders in Yaounde-Cameroon: is perinatal transmission of HBV neglected in Cameroon" BMC Pregnancy Childbirth, 2013; 13:158. https://pubmed.ncbi.nlm.nih.gov/23924215 https://doi.org/10.1186/1471-2393-13-158

25. Djiguiba M. Evaluation de trois recettes dans le traitement traditionnel de l'hépatite B au Mali.Thèsedepharmacie, 2005; 94p.

26. Mouzouvi R, Djègo J, Sehonou J, Lalèyè A, Priuli F, Bigot A" Effet de l'association Combretum micranthum G. Don (Combretaceae) et Cochlospermum tinctorium A. Rich. (Cochlospermaceae) dans la prise en charge de l'hépatite virale B. Série Pharm Journal of African Traditional Medicine, 2014; 17(1):10-14. https://www.google.com/search?q

27. Nergard C, Diallo D, Inngjerdingen K, Michaelsen T, Matsumoto T, Kiyohara H" Medicinal use of Cochlospermum tinctorium in Mali, Anti-ulcer, radical scavenging and immunomodulating activites of polymers in the aqueous extracts of the roots" Journal of. Ethnopharmacol, 2005; 96:255-269. https://pubmed.ncbi.nlm.nih.gov/15588678/ https://doi.org/10.1016/j.jep.2004.09.018

28. Wilasrusmee C, Kittur S, Shah G, Siddiqui J, Bruch D, Wilasrusmee S, Kittur D, (2002)" Immunostimulatory effect of Silybum marianum (milk thistle) extract" Journal of Medicine Sciences,2002; 8:439-443. https://pubmed.ncbi.nlm.nih.gov/12444368

29. Anton G, Hartmut H, Schmidt "Silymarin as supportive treatment in liver diseases: A narrative review"Adv Ther, 2020; 37:12791301. https://pubmed.ncbi.nlm.nih.gov/32065376/

30. Okonkwo U, Aluka A, Ezedinachi E" Effects of silymarin on treatment naïve patients with chronic hepatitis B Infection- A randomized controlled trial" Journal of Infectious Diseases Ther, 2014; 48(6):121-135.https://www.omicsonline.org/open-access-pdfs/effects-of-silymarin-on-treatment-nave-patients-2332-0877.1000168.pdf

31. Yang P, Althage A, Chung J, Maier H, Wieland S, Isogawa M, Chisari FV,"Immune effectors required for hepatitis B virus clearance" Proceedings of National Academy of Sciences, 2010; 107:798802. https://pubmed.ncbi.nlm.nih.gov/20080755/ https://doi.org/10.1073/pnas.0913498107