

# Evaluation of Biochemical and Molecular Parameters of Patients Suffering from Chronic Viral Hepatitis B Treated by a Medicinal Plant Recipe of a Health Care Practitioner in Burkina Faso

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## Abstract

Chronic viral hepatitis B (HBV) remains a major public health problem in Burkina Faso. Since access to diagnostic tests and treatments is limited because of their high cost, the majority of the population turn to traditional herbal treatments. This study aimed to evaluate the effectiveness of a plant recipe called *Hepatib tiben*. It consisted of comparing certain biochemical and molecular parameters of patients infected with HBV that were supported by the recipe. The patients were recruited in Ouagadougou by the traditional health practitioner according to the requirements of the study. Thus 44 patients aged 20 to 61 years and carrier of HBsAg for at least 06 months were treated with *Hepatib tiben*. The tests were performed in the laboratory before and three months after the treatment. ELISA tests were used to confirm the presence of HBsAg and search for anti-HCV antibodies; transaminases, creatinine were quantified by the “Chem 400” automaton and the viral load of HBV by Real-time PCR. The analysis of the results reveals an improvement of the biochemical and molecular parameters of the patients with the following means (ASAT:  $21.02 \pm 9.97$ ; ALAT:  $21.11 \pm 13.27$ ; DNA:  $1571.82 \pm 3990.97$  with  $p = 0.01$  for each). As for HBsAg, its disappearance was observed in 4.55% of patients after treatment. The evaluation of the creatinine parameter explained that

the recipe of plants has a tolerated effect on the kidneys of treated patients. These results, while encouraging, need to be complemented by further research for the development of effective phytomedicine to treat and eliminate this viral hepatitis B virus.

## Keywords

Viral Hepatitis B, Recipe, Plants, Treatment, Burkina Faso

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## 1. Introduction

Hepatitis B is one of the most prevalent human viral infections in the world, alongside HIV, Tuberculosis and Malaria [1]. It shares the same routes of transmission as HCV and HIV, and exposes populations to risks of co-infection with HBV/HIV, HBV/HCV, HCV/HIV [2] [3]. Hepatitis B affects the liver and its chronicity can induce liver fibrosis that can progress to cirrhosis and then Hepatocellular Carcinoma (HCC) which represents the fourth most common cancer in the world and the third causing the greatest number of deaths [4]. The prevalence of this infection is higher in West Africa and is 8% [1]. In Burkina Faso, 1.9 million people live with HBV [5] with an estimated prevalence between 12% - 14.5% [1]. The high cost of antivirals, but also the attachment to some ancestral practices of the populations lead them to resort to traditional medicine to satisfy their health need, although this disease is treated by modern medicine [6]. WHO estimates that 80% of the world's population uses traditional medicine for their health needs. However, since 1994, Burkina Faso has integrated traditional pharmacopoeia into its health system, and scientific research on medicinal plants is encouraged [7]. It is, therefore, necessary to establish scientific bases of plants known to be active against HBV in order to validate their use for an effective and accessible control in the promotion of traditional medicine. We have undertaken the present, to carry out an observational study on patients of a traditional health practitioner treated by a recipe of plants *Hepatib tiben* made with *Phyllanthus amarus*, *Cassia nigricans* and *Balanites aegyptiae* to evaluate its effectiveness against HBV.

## 2. Methodology

### 2.1. Study Setting

The study took place in Ouagadougou, Burkina Faso. Venous samples and analyses (biochemical and molecular) were carried out at the Pietro Annigoni Biomolecular Research Center (CERBA) and the Molecular Biology and Genetics Laboratory (LABIOGENE).

### 2.2. Type of Study

This is an observational study, which took place from December 2021 to May

2023.

### 2.3. Target Population and Conditions of the Study

The target population of the study was patients with HBsAg for more than six months who were followed by a health care practitioner in Ouagadougou. These patients were recruited by the traditional health practitioner on a consultation basis and consecutively over a period of one and a half years. The inclusion criteria were chronic carriage of HBsAg confirmed on the basis of ELISA positivity; to have been screened at least six months before the start of the study. A biological examination was conducted as part of the recruitment process. The examination reports were prepared in collaboration with the traditional health practitioner. Thus, out of 83 patients consulted, only 44 patients who met the inclusion criteria and followed the treatment until the end were selected at the end of the study. An initial checkup was asked of all patients and involved: the search for HBsAg which was carried out by the Immuno-enzymatic ELISA, based on an in vitro immunochromatographic rapid assay, designed for qualitative detection of hepatitis B specific surface antigens in serum [8], the determination of creatinine, ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase) by a biochemical automaton “Chem 400”, and HBV viral load by PCR (Polymerase Chain Reaction) in real temp. Before the viral DNA assay, an extraction was performed using the Qiagen kit following the manufacturer’s protocol. The PCR was then performed with the “primer design™ Ltd Hepatitis B Virus Core Protein Region genesig<sup>R</sup> Advanced kit” according to the following amplification program:

- Enzyme activation, 2 min at 95°C
  - Denaturation, 10s at 95°C
  - Hybridization/Data Collection, 60s at 60°C
- } 50 cycles

After this primary assessment, the treatment was initiated with the *Hepatib tiben* recipe, based on the three plants, by the traditional health practitioner.

### 2.4. Preparation and Treatment Regimen

**Preparation:** The recipe *Hepatib tiben* made from whole plants of *Phyllanthus amarus*, *Cassia nigricans*, leaves of *Balanites aegyptiae* and ingredients, is prepared and packaged in capsules, then in boxes containing 60 capsules, ready for consumption. This step is carried out by the traditional health practitioner.

**Dosage and duration of treatment:** The recipe is prescribed by the traditional health practitioner to patients because of 03 boxes per patient. Each box contains 60 capsules for a dosage of 02 capsules per day (morning and evening). The duration of treatment is 90 days.

**Control tests:** After the treatment thus instituted, controls were carried out to evaluate the effectiveness of the *Hepatib tiben* recipe on the biological parameters (HBsAg, viral load, transaminases, creatinine) of the patients.

**Parameters evaluated:** The efficacy criteria of the treatment were: a decrease or normalization of transaminases, a stability of creatinine as well as a disappearance

of HBsAg and an undetectable viral load.

**Study limitation:** Recommended Inclusion Criteria for Hepatitis Treatment Decisions [9] have not all been assessed. Only criteria used in developing countries to evaluate the effectiveness of plants in the treatment of chronic viral hepatitis, including DNA-HBV, anti-HCV and creatinine PCR tests, were considered. Furthermore, of the 83 patients consulted, fifty (50) patients were initially included and six (06) were excluded during the study due to some non-compliance (consumption of alcohol and fatty foods during treatment, among others) and for others absenteeism at appointments. Despite the small sample size, this study allowed us to make observations and can be supplemented by further in-depth studies.

**Ethics committee:** The study has been approved by the Ethics Committee for Health Research, deliberation No: 2023-06-150.

**Statistical analysis:** The data was entered into the Excel spreadsheet and analyzed with the R and SPSS software. The nonparametric Wilcoxon test was used for comparison analyses to assess the significance between pre- and post-treatment tests,  $p < 0.05$ .

### 3. Results

#### 3.1. Sociodemographic Characteristics

The sociodemographic characteristics available in the traditional health practitioner were the age and sex of the patients. Among the 44 patients included in our study, 21 or 47.73% are male and 23 or 52.27% are female. The sex ratio is 0.91 for women. The majority of patients were aged 20 to 40 years or 65.90%. Bearing of HBsAg was positive in all participants and that of AC anti HCV in 02 patients. The socio-demographic characteristics are summarized in **Table 1**.

**Table 1.** Socio-demographic characteristics.

<b>Sex n (%)</b>	
<b>Men</b>	21 (47,73)
<b>Woman</b>	23 (52,27)
<b>Age (year)</b>	
<b>Mean</b>	36,45 ± 10,45
<b>Interval</b>	20 - 61
<b>HBsAg n (%)</b>	
<b>Positive</b>	44 (100)
<b>Negative</b>	0
<b>AC anti HCV n (%)</b>	
<b>Positive</b>	2 (4,5)
<b>Negative</b>	29 (65,9)
<b>Not specified</b>	13 (29,5)

### 3.2. Biochemical and Molecular Parameters before Treatment

Before starting treatment, HBsAg was positive in all patients included in the study. Only 9.09% of women had creatinine above the norm. Serum activity of transaminases was high in 31.81% with a viral load between 0 - 21 700 000 UL/mL. The values of the biological parameters initially measured are summarized in **Table 2**.

**Table 2.** Results of biochemical and molecular characteristics of patients before treatment.

Parameters	Mean	Standard deviation	Minimum	Maximum
ALAT (UI/L)	25.14	15.51	7	92
ASAT (UI/L)	25.89	9.96	6	63
Creatinine (mg/L)	89.42	23	11.8	136.1
HBV viral load (UL/mL)	497 443	3 270 767.88	0	21 700 000

### 3.3. Biochemical and Molecular Parameters after Treatment

After three months of treatment with *Hepatib tiben* administered by the traditional health practitioner, the biological and molecular parameters were measured and recorded in **Table 3**. Results show a clear improvement in HBV viral load from a mean of 497 443 UL/mL before treatment to 1 571.82 UL/mL after treatment with the plant recipe.

**Table 3.** Results of biochemical and molecular characteristics of patients after treatment.

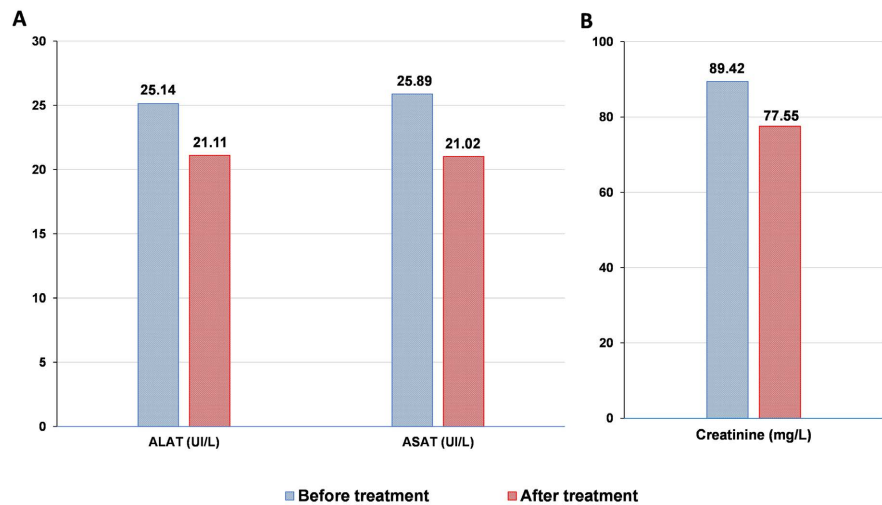
Parameters	Mean	Standard deviation	Minimum	Maximum
ALAT (UI/L)	21.11	13.27	6	71
ASAT (UI/L)	21.02	9.98	11	52
Creatinine (mg/L)	77.55	27.02	57.8	129.3
HBV viral load (UL/mL)	1 571.82	3 990.97	0	21 200

### 3.4. General evolution of Markers

Regarding the general evolution, 02 patients had a favorable state regarding HBsAg, 05 and 15 had an unfavorable state regarding the evolution of transaminases and viral load (**Table 4**) also illustrated through **Figure 1** and **Figure 2**.

**Table 4.** General Changes in Patient Treatment Effectiveness.

Evolution	Stationary		Unfavourable		Favorable		Total	
	N	%	N	%	N	%	N	%
HBsAg	42	95.45	0	0	2	4.55	44	100
ASAT	37	84.09	3	6.82	4	9.09	44	100
ALAT	37	84.09	2	6.82	5	9.09	44	100
HBV viral load	2	4.55	15	34.09	27	61.36	44	100
Creatinine	39	88.63	2	4.55	3	6.82	44	100



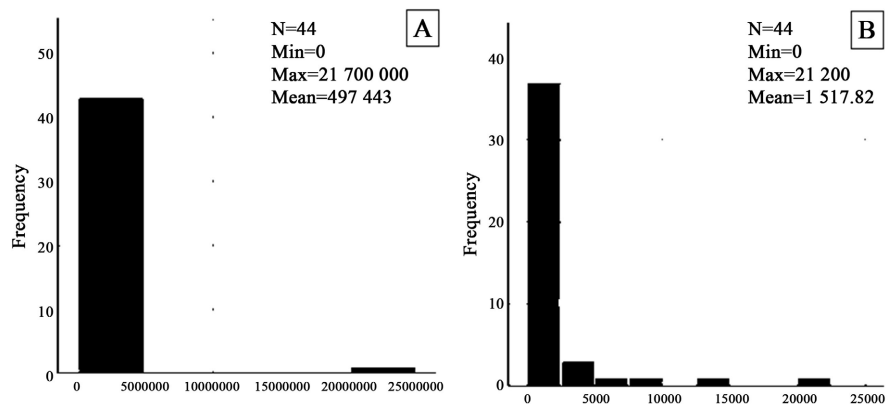
**Figure 1.** Biological parameters before and after treatment. A: ALAT and ASAT. B: Creatinine.

### 3.5. Test Summary Wilcoxon Ranking for Linked Samples

The nonparametric Wilcoxon test was used for HBV DNA comparison analyses before and after treatment. The null hypothesis is rejected.  $P = 0.008$  therefore less than 0.05. There is therefore a significant difference between HBV DNA before and after treatment.

Summary of the hypothesis test				
	Null hypothesis	Test	Sig.	Decision
1	The median difference between Ap-DNA-HBV and Av-DNA-HBV is 0.	Wilcoxon signed rank test of associated samples	0.008	Reject the null hypothesis.

Asymptotic meanings are displayed. The meaning level is ,05.



**Figure 2.** Wilcoxon test. A: Viral load before treatment (Av-DNA-HBV), B: Viral load after treatment (Ap-DNA-HBV).

### 3.6. Other Treatments

In addition, some patients had already used other treatments either traditional or modern (Tenofovir, Entecavir). The results are recorded in **Table 5**.

**Table 5.** Other treatments.

Characteristic	N (%)
No treatment	21 (48)
Unspecified	16 (36)
Tenofovir	4 (9.1)
Traditional treatment	3 (6.8)

#### 4. Discussion

The purpose of this study was to assess the effect of the *Hepatib tiben* recipe, made from whole plants of *Phyllanthus amarus* and *Cassia nigricans*, *Balanites aegyptiaca* leaves and ingredients on some biological markers for monitoring patients with chronic HBV.

The sample of our study is characterized by a female predominance. Indeed, previous studies have shown that women are predominant in Burkina Faso and represent 51.7% of the population [10]. Yet according to the WHO, the transition to chronic hepatitis B is more common in men who are more exposed to complications such as cirrhosis [5]. These results could be explained by the fact that women are more receptive to sensitization and have more access to health centers in the case of prenatal assessments or follow-up of their children for example.

Most of our patients have an age between 20 and 40 years with the average age of 36.45 years (10.45). We see that young people are the most affected by hepatitis. This is not surprising since sexual activity is one of the main routes of transmission of viral hepatitis B, and this age corresponds to the most intense and sometimes uncontrollable period of sexual activity.

Biologically, there was a considerable decrease in viral load, 61.36% of cases and the disappearance of HBsAg was observed in 4.54% of cases or 02 patients. These results are close to those obtained by [11] where the disappearance of HBsAg was observed in 4.17% of cases. A study by Xia *et al.*, found no difference between patients treated with *Phyllanthus amarus* and others with antiretrovirals that aim to decrease viral load [12]. This considerable decrease in viral load and the disappearance of HBsAg in our case could indeed be justified by the fact that the association of *Cassia nigricans* and leaves of *Balanites aegyptiaca* with *Phyllanthus amarus* have greater properties pharmacotherapeutic than *Phyllanthus amarus* alone, or that patients strictly followed the traditional health practitioner's instructions during treatment that were to avoid alcohol and fatty foods, or spontaneous healing of patients. It should also be noted that some had already used other traditional or modern treatments before the start of this study. Indeed, the literature indicates a possibility of seroconversion of HBsAg, after a delay of several months to several years, when HBV DNA is detectable in blood and in the absence of HBsAg. This is called occult hepatitis B [13]. The incidence rate is 2% per year [14]. Randomized trials are recommended on a larger number of patients, including a control group treated with modern drugs (Tenofovir, Entecavir...) and over a long period, to better assess the impact of the Hépatib tiben recipe on the disappearance of HBsAg. In addition, a decrease in transaminases in 14 patients

or 15.9% for ASAT and ALAT, which had values above the norm was observed after three months of treatment. Transaminases were evaluated at 9.09% for ASAT and ALAT respectively. It is slightly appreciable when we analyze the results of Djiguiba *et al.*, in 2005 [15], which after three months of treatment did not observe any significant change. This modification could be associated with a possible antiviral property since the recipe composed of *Phyllanthus amarus*, *Cassia nigricans* and *Balanites aegyptiaca* is a cocktail of tannins that are known for their antiviral properties [16] [17] Cell lysis being at the origin of the increase of transaminases, one could also consider this decrease as being the consequence of an anti-inflammatory property all the more as constituents such as flavonoids, steroids endowed with anti-inflammatory properties are present in the studied plants [18] [19]. The difference between our results and those of others may be explained by a number of variables including extrinsic factors (method of preparation, storage conditions, dosage, dosage form, extraction solvent and dosage) and intrinsic (age, period of harvest of plants and nature of drug). In this study, *Hepatitis tiben* was administered at a dosage of 2 g/day or two capsules of 1g each. In previous studies, a dosage of 10 g/0.5 L water twice daily was adopted [14] and 5 g of leaves/ 1L water/day [11]. In addition, creatinine was assayed in all patients before and after treatment to assess the toxicity of the recipe on renal activity. In our study the biochemical parameter creatinine was unfavorable in only 01 patient probably due to non-compliance with the instructions or the association of *Hepatitis tiben* with other drugs. This proves that the recipe *Hepatitis tiben* does not greatly affect the kidneys.

## 5. Conclusion

It appears from our study that *Hepatitis tiben* which is a recipe made from *Phyllanthus amarus*, *Cassia nigricans*, *Balanites aegyptiae* and ingredients improves the health of patients with chronic HBV by causing a decrease in viral load and also normalization of transaminase activity after 90 days of treatment. It should also be noted that ingestion of this drug does not affect kidney activity. These results shed new light on the pharmacological potential of many traditional medicine and pharmacopoeia plants in Burkina Faso. In view of its effects, this drug can be an alternative to the treatment of chronic hepatitis B in developing countries such as Burkina Faso with a rich and diverse flora. However, it is recommended to extend the study to a higher number of patients and to evaluate all the biological parameters involved in this viral pathology after treatment with *Hepatitis tiben* in order to validate this study.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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