

# Anti-Inflammatory, Viral Replication Suppression and Hepatoprotective activities of Bitter Kola - Lime Juice, - Honey Mixture in HBeAg Seropositive patients

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## Abstract

**Study Background:** Bitter kola (*Garcinia kola*), lime juice, and honey mixture have some traditional but nonscientific applications in the treatment of some infectious diseases due to the phytochemical and phytonutrient components that may have antidiabetic, anti-inflammatory, antipyretic, immunomodulatory, antiatherogenic, antimicrobial, purgative and antiparasitic activities while expression of hepatitis B envelope antigen (HBeAg) is an indication of active hepatitis B virus replication and a high degree of infectiousness. **Aim and Objective:** This work was therefore designed to determine anti-inflammatory response, possible suppression of active viral replication and liver damage in HBeAg seropositive patients who used bitter kola, lime juice and honey mixture. **Materials and Methods:** Out of 39 hepatitis B surface antigen and HBeAg seropositive patients who volunteered themselves for the study only 23 volunteers (23; Male: 13; Female: 10; age 34–56 years) were successfully monitored. The mixture is prepared by mixing grounded bitter kola (30 seeds) with 500 ml of Lime Juice and 500 ml of honey. The mixture was kept for 5 days before administration. The mixture was taken by subjects at 4 divided dose for 2 months using 4 teaspoonfuls per dose. Plasma alanine transaminase (ALT) was measured using COBAS C111 autoanalyzer, plasma interleukin-10 (IL-10), Antibodies against hepatitis C virus (anti-HCV), HBeAg, anti-HBeAg, p24 ag-ab by enzyme-linked immunosorbent assay while acid-fast bacilli and *Plasmodium* were determined in the subjects by microscopic examination of stained smears. **Results:** The frequency of HBeAg (26.1% [6]) in the patients after the use of bitter kola, lime juice, and honey mixture was lower than the frequency of the viral protein (HBeAg 100% [23]) before the use of the mixture. None of the HBeAg seropositive patients studied expressed anti-HBeAg before the use of bitter kola, lime juice, and honey mixture but 56.5% (13) of the patients expressed anti-HBeAg while 17.4% (4) co-expressed HBeAg + anti-HBeAg after the use of the mixture. There was a significant increase in the plasma value of IL-10 and a significant decrease in the plasma value of ALT in the HBeAg seropositive patients after the use of bitter kola, lime juice, and honey mixture ( $P < 0.05$ ). **Conclusion:** Bitter kola, lime juice, and honey mixture could have anti-inflammatory, active viral replication, and liver damage suppression bioactivities in HBeAg seropositive patients as indicated by decrease in HBeAg, expression of anti-HBeAg, decrease in plasma ALT and increase in IL-10 after the use of the mixture.

**Keywords:** Alanine transaminase, anti-hepatitis B envelope antigen, bitter kola, hepatitis B envelope antigen, honey mixture, lime juice, liver damage, viral replication

## INTRODUCTION

Interleukin 10 (IL-10) cytokine is a human cytokine synthesis inhibitory factor and an anti-inflammatory cytokine.<sup>[1-5]</sup> It inhibits the production of pro-inflammatory cytokines.<sup>[1-5]</sup> IL-10 cytokine an anti-inflammatory cytokine enhances B cell survival and proliferation. It stimulates antibody production.<sup>[1-5]</sup> IL-10 cytokine suppresses antigen-presenting cells regarding antigen-presentation capacity.<sup>[1-5]</sup>

Alanine transaminase (ALT) or alanine aminotransferase or serum glutamate-pyruvate transaminase or serum

glutamic-pyruvic transaminase is a biomarker of liver damage because it is mostly found in the liver but it is also in plasma

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and in various body tissues.<sup>[6,7]</sup> It is an important liver enzyme because it catalyzes the transfer of an amino group from L-alanine to  $\alpha$ -ketoglutarate in a reversible transamination reaction to produce pyruvate and L-glutamate.<sup>[6,7]</sup>

Hepatitis B envelope antigen (HBeAg) is a hepatitis B virus protein that indicates hepatitis B virus infection (HBV),<sup>[8]</sup> active viral replication, and infectiousness.<sup>[8]</sup> Hepatitis B virus-infected patients who express HBeAg is highly infectious and can transmit the virus to another person.<sup>[8]</sup> The disappearance of HBeAg in the serum of HBV-infected patients indicates the end of the acute phase and convalescence.<sup>[8]</sup> The antibody to HBeAg is known as anti-HBe or anti-HBe. Expression of anti-HBe or anti-HBeAg indicates HBV infection and clearance of HBeAg.<sup>[8]</sup> It may indicate convalescence and the production of the antibody always leads to decline in viral replication.<sup>[8]</sup>

Hepatitis B surface antigen (HBsAg) in serum indicates HBV infection and current hepatitis B infection. It may be in the serum with or without clinical symptoms.<sup>[8,9]</sup> Expression of antibodies against HBsAg (anti-HBsAg) by HBV patients usually means noninfectious and clearance of HBsAg.<sup>[8,9]</sup>

HBsAg is the first antigen shed into the plasma followed by HBeAg in HBV infection.<sup>[8,9]</sup> Persistence of HBsAg and HBeAg in the serum beyond 6 months indicates chronic infection as the antibodies to these antigens are produced and clear the antigens after 6 months of the expression of the antigens. Antibodies to envelope and surface antigens may indicate convalescence and lesser severity.<sup>[8,9]</sup>

Bitter kola (*Garcinia kola*) seeds are edible commonly eaten to refresh. There are traditional nonscientific claims that it has purgative, antiparasitic, and antimicrobial properties and as such has been used for liver disorders, bronchitis, colic, head or chest colds, and cough. Infectious diseases, like throat infections, hepatitis, infections due to the influenza virus, and other viral diseases.<sup>[10]</sup> The seed has pharmacological effects such as antidiabetic, anti-inflammatory, antipyretic, immunomodulatory, antiatherogenic, antimicrobial, and liver disorders. It has an LD50 of above 5000 mg/kg.<sup>[11]</sup> *Garcinia kola* has both nutritional and pharmaceutical properties because it contains vitamins, minerals, proteins, carbohydrates as well as phytochemicals such as alkaloids, flavonoids, tannins, cyanogenic glycosides, and saponins. The antiviral, anti-inflammatory, antidiabetic, bronchodilator, and antihepatotoxic activities have been attributed to *Garcinia* bioflavonoids component, while its antimicrobial activity has also been reported to be due to polyisoprenylated benzophenone constituent.<sup>[12]</sup>

Honey and lime juice contain antimicrobial, anti-inflammatory, and antioxidant nutritive materials. Honey contains sugars, dietary fiber, Fat, Protein, Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Vitamin B6, Folate (B9), Vitamin C, Calcium, Iron, Magnesium, Phosphorus, Potassium, and Zinc.<sup>[13-16]</sup> Limes are a rich source of Vitamin C (35% per 100 g) which is a potent antioxidant vitamin. In

general, lime juice contains carbohydrates Sugars, Dietary fiber, Fat, Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Vitamin B6, Folate (B9), Vitamin C, Calcium, Iron, Magnesium, Phosphorus and Potassium.<sup>[17-19]</sup> Honey also contains an antibacterial agent known as methylglyoxal and the antimicrobial peptide bee defensin-1.<sup>[13-16]</sup>

This work was therefore designed to determine anti-inflammatory response, possible suppression of active viral replication and liver damage in HBeAg seropositive patients who used bitter kola, lime juice and honey mixture to assess the nonscientific traditional claim in the use of the mixture to treat viral hepatitis B.

## MATERIALS AND METHODS

### Study area

The volunteers were recruited from the Saki-West Local government area located in the Northern part of Oyo State in the South-West geopolitical zone of Nigeria. The local government has primary, secondary and tertiary hospitals and educational institutions. There are more than 20 traditional homes in the area.

### Study population

Out of 39 HBsAg and HBeAg seropositive patients who volunteered themselves for the study only 23 volunteers (23; Male: 13; Female: 10; age 34–56 years) were successfully monitored.

### Preparation and administration of bitter kola mixture

The mixture is prepared by mixing grounded bitter kola (30 seeds) with 500 ml of Lime Juice and 500 ml of honey. The mixture was kept for 5 days before administration. The mixture was taken by subjects at 4 divided dose for 2 months using 4 teaspoonfuls per dose.

### HIVp24 antigen and antibodies to HIV-1 and HIV-2 in human serum

HIVp24 antigen and antibodies to HIV-1 and HIV-2 in human was determined in the serum of each of the subjects using Bio-Rad Genscreen ULTRA HIV Ag-Ab, a qualitative enzyme-linked immunosorbent assay (ELISA) kit.

### Anti-hepatitis C virus enzyme-linked immunosorbent assay

Anti-HCV was detected in the subjects using Bio-Rad Monolisa™ Anti-HCV PLUS Version 3 screening kit.

### Hepatitis B envelope antigen and hepatitis B envelope antibody enzyme-linked immunosorbent assay

HBeAg and anti-HBe tests were determined in the subjects by ELISA using the reagent kit of DIA. PRO Diagnostic Bioprobes Srl Via Columella, Milano– Italy Manufacturer's instructions were strictly followed and applied.

### Human interleukin-10 enzyme-linked immunosorbent assay

Plasma IL-10 was evaluated in the subjects by ELISA using Human ABCAM ELISA Kit.

### Detection of acid-fast bacilli in sputum and identification of *Plasmodium* in blood

Acid-fast bacilli in sputum and *Plasmodium* in blood were identified by the methods described by Cheesbrough.<sup>[20]</sup>

### Alanine transaminase

Plasma ALT was determine using COBAS C111.

### Method of data analysis

The raw data obtained were analyzed for mean, frequency, and standard deviation, Student’s *t*-test and probability at 0.05 level of significance using SPSS IBM 20.0 (New York).

### Ethical consideration

The proposal was reviewed, amendments were made as prescribed and ethical approval was given for the work by Research and Ethical Committee of Oyo State Hospital Management Board (OYSHMB/RE/IX/238).

## RESULTS

The frequency of HBeAg (26.1% [6]) in the patients after the use of bitter kola, lime juice, and honey mixture was lower than the frequency of the viral protein (HBeAg (100% [23]) before the use of the mixture [Table 1 and Figure 1].

None of the HBeAg seropositive patients studied expressed hepatitis B envelope antibody (HBeAb) (anti-HBeAg) before the use of bitter kola, lime juice, and honey mixture but 56.5% (13) of the patients expressed HBeAb while 17.4% (4) co-expressed HBeAg + HBeAb after the use of the mixture [Table 1 and Figure 1].

There was a significant increase in the plasma value of IL-10 and a significant decrease in the plasma value of ALT in the HBeAg seropositive patients after the use of bitter kola, lime juice, and honey mixture [ $P < 0.05$ ; Table 1 and Figures 1 and 2].

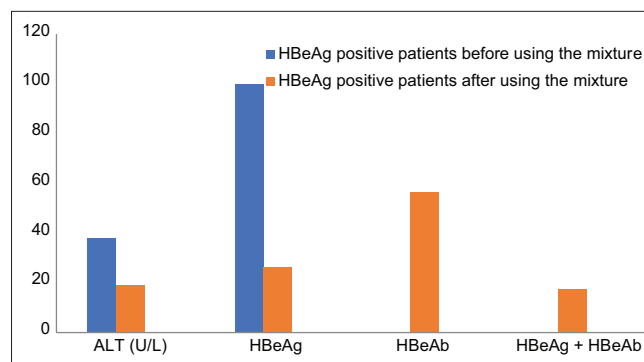
## DISCUSSION

This work was used to determine anti-inflammatory response, possible suppression of active viral replication, and liver

damage in HBeAg seropositive patients who used bitter kola, lime juice, and honey mixture to assess the nonscientific traditional claim in the use of the mixture to treat Viral hepatitis B. The results obtained as therefore as stated and discussed in the following paragraphs.

The frequency of HBeAg (26.1% [6]) in the patients after the use of bitter kola, lime juice, and honey mixture was lower than the frequency of the viral protein (HBeAg (100% [23]) before the use of the mixture which can be attributed to the potential of the mixture to boost immunity for viral clearance of HBeAg. The results also showed that none of the HBeAg seropositive volunteers studied expressed HBeAb before the use of bitter kola, lime juice, and honey mixture but 56.5% (13) of the patients expressed HBeAb while 17.4%(4) co-expressed HBeAg + HBeAb after the use of the mixture.

HBeAg is a hepatitis B virus protein of which the expression in HBeAg seropositive patients indicates active hepatitis B virus (HBV) replication and a very high degree of infectiousness.<sup>[8,9]</sup> Those who volunteered and accepted for this work were those who expressed HBeAg without the antibody (HBeAb), however after 2 months

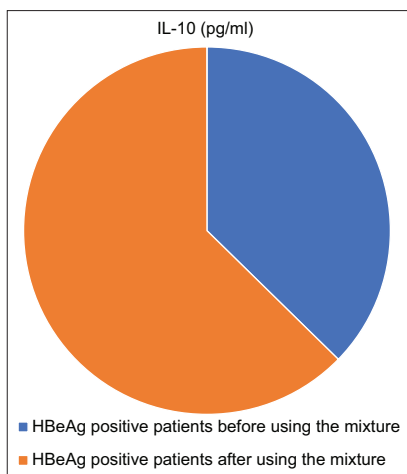


**Figure 1:** Comparative discription of plasma ALT, HBeAg, HBeAb and HBeAg + HBeAb in HBeAg seropositive patients before and after the use of bitter kola, lime juice and honey mixture. HBeAg: Hepatitis B envelope antigen, ALT: Alanine transaminase, HBeAb: Hepatitis B envelope antibody

**Table 1: Plasma alanine transaminase, interleukin-10, hepatitis B envelope antigen, and anti-hepatitis B envelope antigen obtained in the subjects**

	HBeAg Seropositive patients before using the mixture	HBeAg Seropositive patients after using the mixture	<i>t</i>	<i>P</i>
<i>n</i>	23	23		
ALT (U/L)	38±4.0	19±2.0	4.24853	0.03*
IL-10 (pg/ml)	2.8±0.1	4.7±0.2	-8.49706	0.007**
HBeAg	100% (23)	26.1% (6)		
HBeAb	0	56.5% (13)		
HBeAg + HBeAb	0	17.4% (4)		
<i>Plasmodium</i>	Negative	Negative		
AFB	Negative	Negative		
p24 Ag-Ab	Negative	Negative		
Anti-HCV	Negative	Negative		

\*Significant, \*\*Highly Significant. HBeAg: Hepatitis B envelope antigen, ALT: Alanine transaminase, IL: Interleukin, HBeAb: Hepatitis B envelope antibody, AFB: Acid fast bacilli, HCV: Hepatitis C virus



**Figure 2:** Comparative discription of plasma IL-10 in HBeAg seropositive patients before and after the use of bitter kola, lime juice and honey mixture. HBeAg: Hepatitis B envelope antigen, IL-10: Interleukin

of the use of bitter kola, lime juice, and honey mixture majority of the volunteers expressed HBeAb though few co-expressed HBeAg with HBeAb. Expression of HBeAb or anti-HBeAg in HBV patients is an indication of clearance of HBeAg, decreased infectiousness, lack of active hepatitis B virus replication, convalescence, and immunity to HBV.<sup>[8,9]</sup> The results obtained after the use of bitter kola, lime juice, and honey mixture regarding the expression of anti-HBeAg and HBeAg clearance<sup>[8,9]</sup> can be attributed to the phytochemical and the phytonutrient constituents of the mixture as bitter kola (*Garcinia kola*) contains alkaloids, flavonoids, tannins, cyanogenic glycosides, saponins, *Garcinia* bioflavonoids component and polyisoprenylated benzophenone constituents that are responsible for antiviral, anti-inflammatory, antidiabetic, bronchodilator, and antihepatotoxic and antimicrobial properties of bitter kola seeds.<sup>[10-16]</sup> Another associating factor is that lime juice is highly rich in vitamin C (35%) which is a potent antioxidant, honey also contains antioxidant vitamins and minerals, antibacterial agent known as methylglyoxal, and the antimicrobial peptide bee defensin-1.<sup>[10-16]</sup>

Antiviral and anti-inflammatory properties of bitter kola due to *Garcinia* bioflavonoids component can also be attributed to HBeAg clearance and lack of active viral replication.<sup>[10-16]</sup> There was a significant increase in the plasma value of IL-10 and a significant decrease in the plasma value of ALT in the HBeAg seropositive patients after the use of bitter kola, lime juice, and honey mixture.<sup>[10-16]</sup>

IL-10 is an anti-inflammatory cytokine that inhibits viral replication, tumorigenesis, and production of pro-inflammatory cytokine.<sup>[1-5]</sup> It also stimulates the production of antibodies which in this case is anti-HBeAg for viral clearance of HBeAg leading to suppression of viral replication.<sup>[1-5]</sup> In this study, a significant increase in IL-10 after the use of the mixture can be attributed to the phytochemical and phytonutrient components of the mixture as discussed earlier.<sup>[10-16]</sup>

Furthermore, ALT is a biomarker of liver damage whose plasma level increases as a result of hepatocellular damage.<sup>[6,7]</sup> Decrease in plasma ALT in this work after the use of the mixture indicates suppression of liver damage due to anti-inflammatory and ant-oxidative properties of the mixture attributable to the phytochemical and phytonutrient components of the mixture as discussed earlier.<sup>[10-16]</sup>

## CONCLUSION

Bitter kola, lime juice, and honey mixture could have anti-inflammatory, active viral replication and liver damage suppression bioactivities in HBeAg seropositive patients as indicated by decrease in HBeAg, expression of anti-HBeAg, decrease in plasma ALT and increase in IL-10 after the use of the mixture.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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