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Lipid peroxidation and antioxidants during treatment of hepatitis -b with *phyllanthus amarus*

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ABSTRACT

Hepatitis-B is a global public health problem in India, and responsible for major chunk of morbidity and mortality. The present study focuses on effect of *phyllanthus amarus* therapy for protection of liver in hepatitis-B through investigating liver profile enzymes, antioxidant enzymes, antioxidant vitamins and lipid peroxidation (LPO). The study consists of 65 clinical diagnosed hepatitis-B patients ranging in between age group 25-60 years. The control group includes 65 ages and sex matched normal healthy persons. Oxidative stress was assessed by estimating lipid peroxides as MDA. The parameters like serum bilirubin, total proteins and activity of liver profile enzymes were done. Activity of enzymatic antioxidants, superoxide dismutase [SOD], glutathione peroxides [GPx], catalase and levels of non-enzymatic antioxidant vitamin E and vitamin C was measured in plasma or erythrocytes. Methods used in the study are mainly enzyme kinetics by auto analyzer and by turbidimetry. Plasma LPO levels were significantly high but activity of SOD, GPx, catalase and levels of vitamin E and vitamin C were significantly lowered in hepatitis- B on comparison with controls. After *phyllanthus amarus* therapy for 4 weeks and 8 weeks plasma LPO levels were significantly decreased and activity of SOD, GPx, catalase and vitamin E and vitamin C were significantly increased in hepatitis-B. The present study concludes that, hepatitis-B increases oxidative stress and may be playing an important role in hepatic cell damage and pathogenesis of hepatitis-B. This study strongly suggests that the therapy with *phyllanthus amarus* increases antioxidants and reduces lipid peroxidation of hepatic cellular and intracellular membranes and protects liver damage due to free radicals in hepatitis-B.

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1. Introduction

Hepatitis-B is a global public health problem in our country, and responsible for major chunk of morbidity and mortality. It occurs endemically and sporadically through the world, depending on the infelicity of infection. It accounts nearly 90% of all jaundice and hepatocellular injury [1]. It has become problem for physicians, community and government in many part of India [2]. 450 million persons are carrier of hepatitis - B virus in the world today out of which 70% reside in area and western pacific [3, 4].

Transmission of infection from HBV carrier mother to her Para neonate, house hold contacts with HBV arriver, hemodialysis, exposure to infected health care workers, tattooing body piercing, sexual activity, blood transfusion etc.

Use of herbal drugs in the treatment of liver diseases has been a long tradition especially in esteem medicine [5,6]. *Phyllanthus* is

used as ayurvedic medicine for over 2000 years and has a wide number of traditional uses including internal use for jaundice, gonorrhoea, frequent menstruation – hepatitis and diabetics [7-9].

Present study is an attempt to investigate the effect of liver regeneration by therapy of *phyllanthus amarus*, assessed by lipid peroxidation and antioxidant defense system and liver profile enzymes. Lipid peroxidation is one of the cellular pathways involved in oxidative damage.

2. Materials and Methods

The present study was carried out with 65 clinically diagnosed hepatitis-B patients ranging in between age group 25 – 60 years from Government Medical College Hospital Miraj, Civil Hospital Sangli and Corporation Ayurvedic Hospital Sangli. Patients having obvious malignancy, hepatic diseases associated renal disease, lung diseases, thyroid diseases, gastrointestinal diseases, alcoholic diseases, tobacco chewers and smoker's cardiac disease and addiction history of alcohol or smoking were excluded. The patients with co-existing other liver disorders were also excluded.

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Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients, and their presence correlates with the development of complications. Hence the present study was undertaken to assess the extent of lipids, hCRP, Hcy, lipid peroxidation and the status of the antioxidant defense system in patients with type 2 diabetes with and without microalbuminuria compare with normal healthy subjects. Written consent was obtained from all enrolled patients as well as controls. For this study the institutions ethical committee approval was taken. All the patients were followed up every week for a period of ten weeks. At each follow up visit, any information about adverse events and a symptomatic evaluation was conducted, which was followed by through the clinical examination. The efficiency of the treatment was judged by the amelioration in subjective symptoms physical and full medical examination and objective signs as well as the degree of improvement in various function tests. Diagnosis of hepatitis – B was done by physicians and conformed by senior physician of Government Medical College Hospital Miraj. Diagnosis of hepatitis – B done with the help of medical history, physical, clinical, hematological and biochemical examinations included test for viral marker. Age and sex matched 65 normal healthy persons as per International Federation of Clinical Chemistry (IFCC) guidelines were included by excluding history of alcohol or smoking as control subjects.

2.1. Sample Collection:

About 10 ml of fasting venous blood samples were collected under sterile condition from hepatitis-B patients before starting any therapy and from normal healthy subjects. After five weeks and ten weeks therapy 10 ml of fasting venous blood samples were collected from hepatitis-B patients under sterile condition. 5 ml blood was taken in sterile dry and acid washed ethylenediamine tetra acetic acid (EDTA) bulbs and 5 ml blood in plain bulbs. Plasma was separated by centrifuging the blood at 3000 rpm for 20 minutes at 40c. This plasma was used for estimation of malondialdehyde (MDA) and vitamin E. The packed cells were used for the analysis of vitamin C, SOD, catalase and GPx. The sera separated were used for the investigation of parameters like total bilirubin, total proteins and activity of enzymes SGPT [10], SGOT [11], ALP [12], GGT [13], 5' NTP [14]. All these parameters were estimated in healthy subjects and all cases before and after the therapy with *phyllanthus amarus*. At the end of five weeks and ten weeks, changes in these parameters from base line values were taken. MDA was determined as the measure of thiobarbituric acid reactive substances (TBARS) [15].

Erythrocytes ascorbic acid levels were estimated by the method of Tietz [16]. Plasma separated was used for estimation of vitamin E by the method of Baker H et al [17]. SOD was determined in the hemolysate by the method of Mishra and Fridovich based on the inhibition of autoxidation of epinephrine to adrenochrome at pH 10.2 [18]. Catalase activity was measured by the method of Beer & Seazer [19] and GPx activity by Paglia and Valentine in erythrocytes [20].

2.2. Statistical analysis

All values are presented as mean \pm SD. Statistical significance was analyzed by Students' 't' test.

3. Results

The levels of lipid peroxidation, vitamin E, vitamin C and activity of SOD, catalase and GPx are presented in the table-1. The levels of lipid peroxidation were significantly higher in hepatitis-B when compared with controls. The levels of vitamin E, vitamin C and activity of antioxidant enzymes SOD, GPx and catalase were

significantly lower in hepatitis-B patients.

The levels of total bilirubin and the activity of liver profile enzymes SGPT, SGOT, ALP, GGT and 5' NTP were significantly increased in hepatitis-B in comparison with control subject (Table-2).

Table 1. Lipid peroxidation and antioxidants in hepatitis-B before and after therapy of *phyllanthus amarus*.

Parameter	Controls (n=65)	Hepatitis-B before therapy (n=65)	Hepatitis-B after 5 weeks therapy (n=65)	Hepatitis-B after 10weeks therapy. (n=65)
LPO n moles/ml.	2.34 \pm 0.68	6.18 \pm 0.62	4.18 \pm 0.62*	2.80 \pm 0.74*
SOD U/gm of Hb.	3.88 \pm 0.80	2.40 \pm 0.70	2.70 \pm 0.38*	3.01 \pm 0.20*
GPx U/gm of Hb.	55.58 \pm 5.50	17.75 \pm 0.25	22.2 \pm 3.00*	39.40 \pm 4.06*
Catalase n mole/ H ₂ O ₂ decomposed /min	640 \pm 115.0	521 \pm 98	570 \pm 60.0*	678 \pm 0.59*
Vitamin E mg/dl.	7.86 \pm 2.52	5.90 \pm 1.43	6.15 \pm 1.58*	6.65 \pm 2.50*
Vitamin C mg/dl.	1.66 \pm 0.46	0.85 \pm 0.10	0.96 \pm 0.17*	1.23 \pm 0.26*

Values are expressed in mean \pm SD, n= Number of observations.

* Indicates P < 0.01 when compared with before therapy.

Table 2. Changes in activity of liver profile enzymes before and after therapy in hepatitis-B patients

Parameter	Controls (n=65)	Hepatitis-B before therapy (n=65)	Hepatitis-B after 5 weeks therapy (n=65)	Hepatitis-B after 10 weeks therapy (n=65)
Total bilirubin(mg/dl)	0.58 \pm 0.02	5.00 \pm 2.95	3.30 \pm 1.50*	1.20 \pm 0.9*
Total protein (g/dl)	7.25 \pm 0.5	6.60 \pm 0.55	6.95 \pm 0.2*	7.15 \pm 0.25*
SGPT (IU/L)	30.92 \pm 7.30	312 \pm 78	225 \pm 61*	122 \pm 69.2*
SGOT (IU/L)	19.32 \pm 0.51	308 \pm 102	154 \pm 48*	62 \pm 32.0*
ALP (IU/L)	114 \pm 4.44	218 \pm 2.1	165 \pm 4.4*	128 \pm 4.31*
GGT (IU/L)	28.92 \pm 0.78	129 \pm 2.60	82 \pm 2.56*	47.41 \pm 3.30*
5' NTP (IU/L)	16.50 \pm 2.58	22.27 \pm 1.52	18 \pm 1.41*	16.53 \pm 2.23*

Values are expressed in mean \pm SD, n= Number of observations.

Indicates * P < 0.01.

4. Discussion

Lipid peroxidation is used as an indicator of oxidative stress in cell and tissues. Thiobarbituric acid reactive substances, malondialdehyde (MDA) the indicator of lipid peroxidation were significantly elevated in hepatitis-B. There is association between increased levels of MDA and hepatitis-B. Free radicals might be attack on unsaturated fatty acids in membrane and organelles to produce lipid peroxides. This may cause decrease in membrane permeability. Thus change in membrane permeability causes cellular damage and necrosis in hepatitis-B.

The activities of liver profile enzymes were significantly increased in hepatitis-B. These enzymes are localized in the cell cytoplasm and cell mitochondria as well as found in bile. Elevated serum enzymes like SGPT, SGOT, ALP, 5' NTP and GGT are indicative to cellular damage and loss of functional integrity of cell membrane in liver. Damage of liver cells by hepatitis-B causes leakage of cellular enzymes into serum [21]. After five weeks and ten weeks therapy of *phyllanthus amarus* there was significantly decreased activity of liver profile enzymes including the reduction in liver cell damage.

The increased concentration of bilirubin and significant rise activity of liver profile enzymes could be taken as an index of liver damage. After five weeks therapy of *phyllanthus amarus* there was significant decrease in LPO and it resumes to normal after ten weeks. Effect of hepatitis-B showed increased oxidative stress by decreasing non-enzymatic antioxidants vitamin E and vitamin C and increasing LPO. In hepatitis-B increased oxidative stress may increase consumption of vitamin E and C. Vitamin E traps free radicals and interrupts the chain reactions that damage the cell [22]. Thus in hepatitis-B, there is increased utilization of vitamin E and vitamin C due to oxidative stress.

SOD is an important antioxidant enzyme having scavenging effect against superoxide anion and catalase is responsible for detoxification of H_2O_2 produced by action of superoxide dismutase and inhibits formation of superoxide radicals [23]. Due to increased oxidative stress there may be increased utilization of enzymes in hepatitis-B to balance the decreased activity of antioxidant enzymes by oxidation through reactive free radicals. Thus the activity of SOD, GPx, and catalase may decrease in hepatitis-B. Decreased antioxidant enzymes and vitamin C and vitamin E might be causing oxiradical-mediated injury and thus may contribute to liver damage. After ten weeks therapy with *phyllanthus amarus* in hepatitis-B decreased levels of vitamin E and vitamin C has been comes to near normal. This indicates that the utilization of vitamin E and vitamin C is decreased and this may be responsible for raised levels of Vitamin E and vitamin C. Increased activity of antioxidant enzymes and vitamins in hepatitis-B indicate that there might be regeneration of liver cells after therapy, which helps in curing hepatitis-B.

Phyllanthus amarus can detoxify the free radicals and has an antioxidant activity [24]. It has been shown to increase protein biosynthesis [25] and increase the rate of regeneration of necrosed cells [26].

5. Conclusion

This study concludes that hepatitis-B increase oxidative stress and may be playing an important role in hepatic cell damage and play a role in pathogenesis of hepatitis. The *phyllanthus amarus* therapy is found to be equally effective in patients of hepatitis-B. The present study strongly suggests that the therapy with *phyllanthus amarus* increases various antioxidants and reduces lipid peroxidation of hepatic cellular and intracellular membranes. Thus *phyllanthus amarus* protects liver damage due to free radicals in hepatitis-B.

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