Research Journal of Pharmaceutical, Biological and Chemical Sciences

Clinical study of hepatoprotective drug Phyllanthus amarus

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ABSTRACT

Numerous medicinal plants and their formulations are used for liver disorder in Ethno medical practice and in traditional system in India. Indigenous plant Phyllanthus amarus was selected for clinical investigation of hepatoprotective activity. The ability of whole dried drug powder of Phyllanthus amarus (a traditionally used in the treatment of Jaundice) was tested for hepatoprotective activity on 107 patients who suffering from liver disease. The powder of the herb was given thrice a day (morning, noon and night, 3 gm each time) orally with water for 30-45 days depending on the severity of the disease. The patients were evaluated for the changes in biochemical markers like SGPT, Billirubin and Haemoglobin on day zero, seven, fourteen, twenty one, twenty eight and forty two. There was significant decrease in SGPT, Billirubin and increase in haemoglobin.

Key words: Phyllanthus amarus, Clinical Studies, Hepatoprotective.

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INTRODUCTION

Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus [1]. The agent should protect against such damage, especially of one which facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue[2]. There is no modern remedy for liver diseases which are so prevalent in the population. The treatment is mainly symptomatic [2].

The antihepatotoxic activity of the drug Phyllanthus amarus which have been studied on the animals, but not on human patients. The marketed herbal formulation incorporating potent hepatoprotective drug Phyllanthus amarus are also thought worth to investigate for their study on patient who suffering from hepatitis [3, 4].

MATERIAL AND METHOD

The plant of Phyllanthus amarus was collected in the month of August 2006 from fields of a village Dugarwada in Modasa Taluka in Sabarkatha District (Gujarat) where it is growing wild. The herb was authenticated from Dr. H.B. Singh, Scientist F & Head, Raw Materials Herbarium & Museum, Council of Scientific and Industrial Research (CSIR), NISCAIR, New Delhi. (Date: 28-07-08, Ref. 1031/62)

The ability of whole dried drug powder of Phyllanthus amarus was tested at Sapan Hospital, Bayad, Dist-S.K., Gujarat, for hepatoprotective activity on 98 patients who were suffering from liver disease, their distribution of patients with age and sex shown in Table no.1. The powder Phyllanthus amarus was given thrice a day (morning, noon and night, 3 gm each time) orally with glucose to the liver damage patients for one, two, three, four and six weeks and treatment was continued until recovered. Pathological parameter like SGPT, Billirubin and Hemoglobin were monitored during the treatment. SGPT levels expressed in U/ml, Billirubin expressed in mg% and Hemoglobin expressed in gm%.

The reagents used in clinical investigations were collected from Span Diagnostic Ltd, Shivam Surgical, Ahmadabad for estimation of SGPT, Billirubin and Haemoglobin.

Estimation of SGPT [5, 6]

Reagent 1: Buffered alanine α-KG substrate.
Reagent 2: DNPH color reagent.
Reagent 3: sodium hydroxide, 4N.
Reagent 4: Working pyruvate standard, 2mM.
Solution 1: One ml of Reagent No.3 was diluted to 10 ml with distilled water. Reagent 1, 2 & 4 are ready for use as such.

Reagent 1(Buffered alanine α-KG substrate) 0.5 ml taken in test tube. It was incubated for 37^oC for 5 min. fasted serum 0.1 ml was added to the test tube. It was mixed well and incubated for 37^oC for 30 min. Reagent 2: DNPH color reagent 0.5 ml was added to the above test tube. It was allow to stand at room temperature for 20 min. Solution 1, 5 ml was added to the solution of the test tube. It was mixed well and allowed to stand for 10 min. the absorbance of the solution was measured 505 nm using water as blank.

Estimation of Billirubin [7]

The estimation of total and direct billirubin is of importance for diagnosis, differentiation and follows up of jaundice. The serum levels of unconjugated billirubin rises in the cases of hemolytic jaundice. Whereas conjugated serum billirubin levels rises in the cases of obstructive jaundice. Hepatic jaundice is characterized by simultaneous rise in both, conjugated and unconjugated serum billirubin levels.

Reagent A: Total billirubin reagent.
Reagent B: Direct billirubin reagent.
Reagent C: Sodium nitrite reagent.
Reagent D: Artificial standard C = 10mg% billirubin.

All reagents in the kit are ready to use as such.

For total billirubin estimation 3 ml of reagent A and 0.1 ml of reagent C were mixed by inversion and waited for 30 seconds. Fasted serum 0.15 ml was added. The content was mixed well and incubated for 37°C for 5 min. absorbance was read at 540 nm using water as blank.

For direct billirubin estimation 3 ml of reagent B and 0.1 ml of reagent C were mixed by inversion and waited for 30 seconds. Fasted serum 0.15 ml was added. The content was mixed well and incubated for 37°C for 5 min. absorbance was read at 540 nm using water as blank.

The absorbance of the reagent 4 (artificial standard) was read directly against distilled water. The standard once used was discarded. Serum Billirubin in mg%

$$\text{Total Billirubin(A)} = \frac{\text{Absorbance of T - Absorbance of TB}}{\text{Absorbance of Standered}} * 10$$

$$\text{Direct Billirubin (B)} = \frac{\text{Absorbance of D - Absorbance of DB}}{\text{Absorbance of Standered}} * 10$$

Where T= Total billirubin, TB= Total billirubin blank, D= Direct billirubin, DB= Direct billirubin blank.

Determination of Haemoglobin [9]

The graduated diluting tube and the micropipette are cleaned thoroughly and dried. The graduated diluting tube is filled with N/10 HCl up to the mark 2 gm or till the micropipette touches the level of acid in the tube. The finger is cleaned with 70% alcohol and it is pricked to obtain a drop of blood. First drop is wiped out. Second drop is sucked in the micropipette up to the mark 20cmm. The blood is immediately deposited at the bottom of the graduated tube. The pipette is rinsed two to three times in HCl. The blood is mixed with the help of stirrer and then solution is allowed to stand for 10-15 minutes so that all Haemoglobin is converted into acid haematin. Then mixture is diluted with distilled water. Distilled water is added drop by drop and every time it is stirred till the exact match with standard glass tubes is obtain and the scale is read on the side of tube.

Statistical analysis

Result of biochemical estimation SGPT, billirubin and Haemoglobin were reported by Mean, S.D, SEM and Median. For determination of significant P value inter group difference of each parameter was analyzed separately. One way analysis of variance P value was carried out by Graph Pad statistics software.

RESULT AND DISCUSSION

The mean SGPT values of the group on zero day is considered as 100%. In comparison with zero week, SGPT level are recovered on first week 46.19%, on second week 64.96%, on third week 79.57%, on fourth week 87.65% and sixth weeks 91.15% respectively which is shown in figure no 1. The P value is < 0.0001, which is considered as highly significant is shown in Table no 2.

The mean Billirubin values of the group on zero day is considered as 100%. In comparison with zero week, billirubin levels are recovered on first week 40.67%, on second week 54.23%, on third week 69.49%, on

TABLE 1: Distribution of patients with Age and Sex.

PHYLLANTHUS AMARUS			
Age	Patients (107)	M/F	Patients
0-15	9	M	6
		F	3
16-30	33	M	27
		F	6
31-45	41	M	26
		F	15
46-60	17	M	9
		F	8
61-ABOVE	7	M	5
		F	2

Where M – Male, F – Female

TABLE 2: P value of SGPT for Phyllanthus amarus.

Duration in week	SGPT value of the group	Standard Deviation	Standard Error of Mean	Median
Initial	747.94	771.02	74.192	535.00
First	402.00	469.50	45.178	210.00
Second	262.13	291.23	32.561	170.00
Third	152.84	144.42	20.027	95.00
Fourth	92.391	71.229	14.540	82.00
Sixth	66.250	19.486	8.714	66.25

The P value is < 0.0001, considered extremely significant.

TABLE 3: P value of Billirubin for Phyllanthus amarus.

Duration in week	Billirubin value of the group	Standard Deviation	Standard Error of Mean	Median
Initial	5.940	3.744	0.3602	4.600
First	3.515	2.804	0.2698	2.700
Second	2.720	2.226	0.2489	2.000
Third	1.847	1.339	0.1856	1.150
Fourth	1.257	0.6261	0.1278	1.000
Sixth	1.050	0.1118	0.0500	1.050

The P value is < 0.0001, considered extremely significant.

TABLE 4: P value of Haemoglobin for Phyllanthus amarus.

Duration in week	Haemoglobin value of the group	Standard Deviation	Standard Error of Mean	Median
Initial	10.896	1.336	0.1286	10.9
First	11.085	1.332	0.1282	11.043
Second	11.166	1.358	0.1518	11.133
Third	11.135	1.374	0.1906	11.168
Fourth	11.387	1.211	0.2472	11.439
Sixth	11.5	1.005	0.4494	11.5

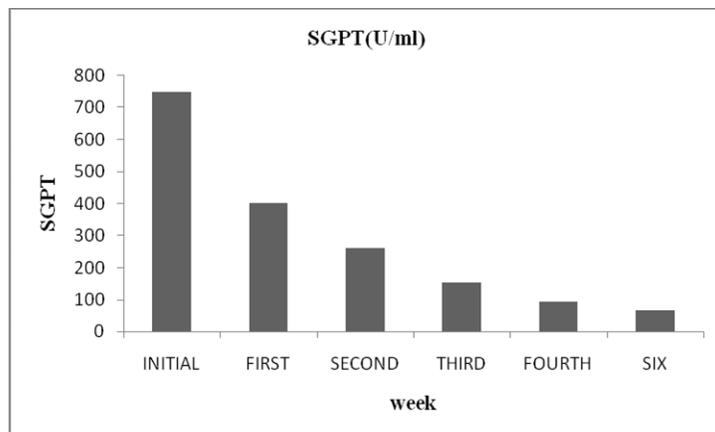The P value is 0.5271, considered not significant.

FIGURE 1: SGPT v/s week. Column graph showing value of SGPT for different week using Phyllanthus amarus.
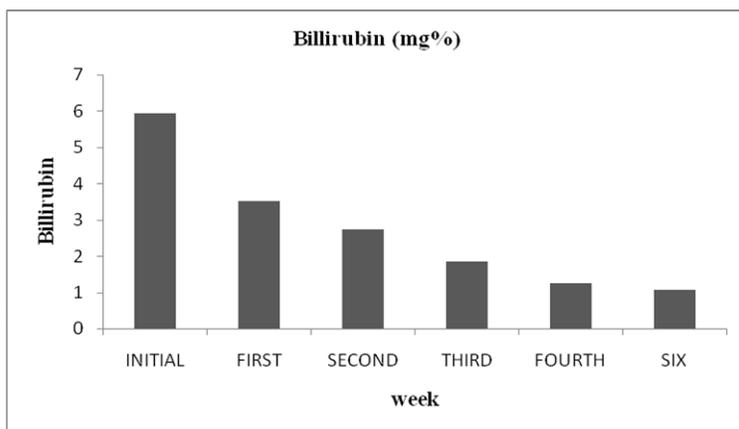

FIGURE 2: Billirubin V/S Week. Column graph showing value of Billirubin for different week using Phyllanthus amarus.
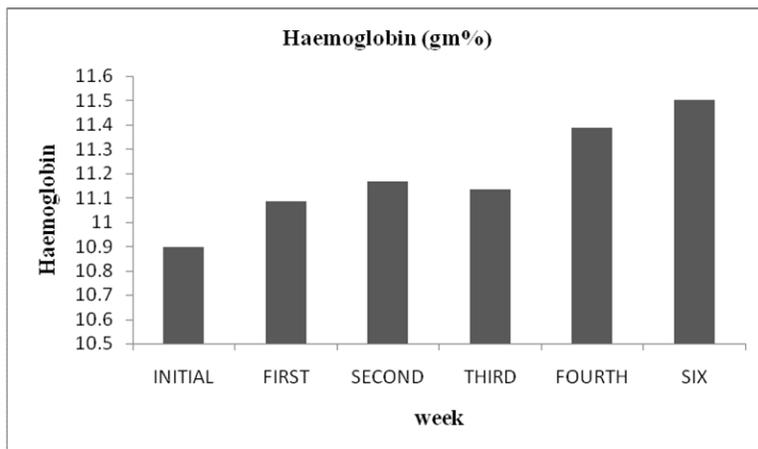

FIGURE 3: Haemoglobin V/S Week. Column graph showing value of Haemoglobin for different week using Phyllanthus amarus.

fourth week 79.66% and on sixth weeks 83.05% respectively is shown in figure no 2. The P value is < 0.0001, which is considered as highly significant is shown in Table no 3.

The mean Hemoglobin values of the group for zero day is considered. As comparison with zero week, haemoglobin level increase on first and second week, on third week haemoglobin level decreases slightly but in comparison with zero week it is increased. On fourth week and sixth weeks increased in hemoglobin respectively is shown in figure no 3. The P value is insignificant for haemoglobin is shown in Table no 4.

CONCLUSION

In patients with damaged liver the treatment with Phyllanthus amarus herb shows decrease in SGPT and Billirubin level with extremely significant P value is <0.0001, while there is an improvement in haemoglobin level. The powder of Phyllanthus amarus shows potent hepatoprotective activity. The Phyllanthus amarus could become helpful for patients with damaged liver possibly by reducing SGPT, Billirubin level. It increases haemoglobin level and possibly improves in life style of such patients.

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