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Study of *Phyllanthus Amarus* Plant Extract and Newly Synthesized Analogue of *Pyllanthin* on Induced Hepatotoxicity in *Albino Rat*

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Abstract

Phyphyllanhtus amarus (Bhuiamala) is medicinally important plant. The literature survey reveals that this plant controls the adverse effect of hepatitis in human being. The extracts isolated from various parts of phyllanthus amarus and newly synthesized analogue of active ingredient i.e. phyllanthin were tested for their curative impact on cythion induced activities in albino rats with special reference to blood serum (VLDL, LDL, and HDL) hepatotoxicity. The effects of intraperitonial administration of test plant extract and snewly synthesized analogue were studied on cythion induced blood serum hepato-toxicity. The blood serum VLDL, LDL and HDL were estimated in order to assess the liver functions by established procedures. Biochemical observations were supplemented with histological examination of liver section. It is evident from the results that levels of various serum lipoproteins were altered in cythion treated animals than control group animals. There was small but significant decrease in the concentration of total serum lipids which may largely due to the reduction of the total lipid concentration in the VLDL, LDL and HDL. However the altered lipoprotein concentration levels were restored almost to the level of their normalcy in Phyphyllanhtus amarus (Bhuiamala) and newly synthesized analogou of active ingredient i.e. phyllanthin treated animals.

Keywords:- Phyphyllanhtus amarus (Bhuiamala), albino rats.

Introduction

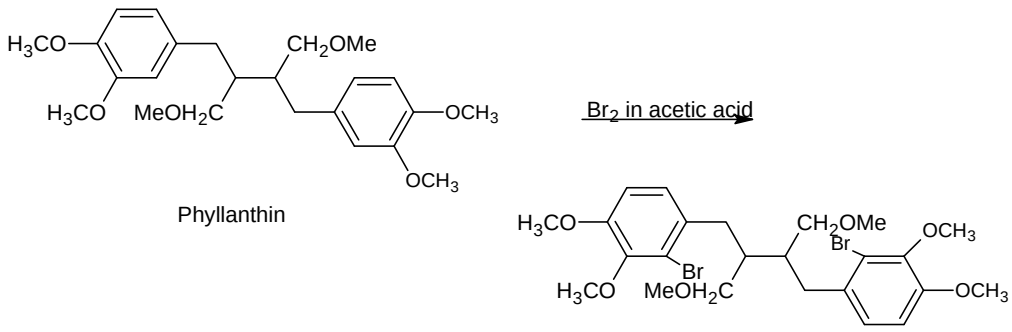
Hepatic diseases is a major public health problem in the developed as well as developing countries. This is due to a wide spread use of pesticides in every field of life that become alarmingly hazardous to human health. Most of the pesticides cause metabolic transformations in the body of higher animals and the main site of metabolism is the liver[1,2]. Any metabolic disturbance in the liver produces characteristic hepatic disease.[3-8] These metabolic disturbances are mainly responsible for physiological and chemical alterations in the liver functions. In fact the use of pesticides and their interactions with liver and its most important bio-constituent lipoprotein is the root cause of many diseases and fatty liver. The major form in which triglycerides released by the liver is very low density lipoproteins (VLDL)[9]. In the circulation of low density lipoprotein (LDL), such release was found in a considerable amount.

Phyphyllanhtus amarus (Bhuiamala): Phyllanthin is one of the active ingredients of *Phyphyllanhtus amarus spp.* It has been reported that the heterocyclic compounds have a broad spectrum of biological activity with special references to curing impact on hepatotxicity.[10] The paucity of data in the study of

hepatoprotective effects of *Phyphyllanhtus* motivated us to undertake the study of test plant extract and analogue prepared on cythion induced hepatic malfunctioning in *albino rats.* We report herewith the synthesis of bromosubstituted phyllanthin as an analogue on its reaction with bromine in acetic acid. The effects of this compound and crude extract of test plant have been studied on cythion induced activities in *albino rats.*

Experimental:-

Phyllanthin (0.01 mol) ingredient extracted from test plant Phyllanthus amarus was dissolved in Glacial acetic acid (2 ml). To this solution bromine in acetic acid reagent was added drop by drop with constant starring. After complete addition of reagent, the reaction mixture was allowed to stand for half an hour. The solid product thus obtained was filtered and washed with a little petroleum ether to get the analogue. It gives yellowish brown layer test with CCl_4 and yellow precipitate with $AgNO_3$ solution thereby confirming the presence of bromine in the product.



Phyllanthin

Bromo derivative of phyllanthin

The spectral analysis of bromo derivative of phyllanthin : **IR (nujol) in cm^{-1} :-** 2916(C-H stre.), 2850.6 (C-H stre.),1168.8 (C-O-C stre.),723.3(C-Br stre.);**UV-VIS ($CHCl_3$)** λ_{max} 370 nm corresponding to n→π*transition; **PMR($CDCl_3$)**:-δ6.8-7.4(m,4H,Ar-H),δ4.5(s,6H,-OCH_3),δ3.5(s,6H,-OCH_3),δ2.5(s,6H,C-OCH_3),δ2.1(d,4H,-CH_2), δ1.5(s,4H,Ar-CH_2-),δ1.4 (m,2H,-CH-). Melting points recorded are uncorrected and their purity was tested by TLC on microscopic slides with silica gel- G layers in benzene. From elemental analysis, chemical properties and spectral data, the compound (2b) assigned the structure bromo-phyllanthin.

Materials and Methods

The healthy *albino rats* of 6-8 weeks of age and 100-200 gm body weight were obtained from Dr. Punjabrao Deshmukh Medical College, Amravati and maintained in suiTable environment. They were supplied commercial pelleted diet and water ad libitum. The animals were divided into four groups (ABCD). The animals of group A were fed on stock diet and used as control species. Animals of group B

were given cythion pesticide intraperitionially (40SD/Kg body wt/day) for one week. Animals of groups C were given newly synthesised crude extract separately. Animals of group D were given analouge (bromo phyllanthin) in conjugation with cythion. The total period of observation was ten weeks.

The blood samples were collected from animals of group ABCD and left to clot at room temperature for at least 30 minutes to remove the clot and cell debries to perform following analysis.

i) Isolation of LDL and VLDL by precipitation with heparin and $MnCl_2$:

To 5ml of serum were added 0.2ml of 5% heparin solution and 0.25 ml of $MnCl_2$ solution. A ppt. appeared immediately. The reaction mixture was centrifuged for 10 minutes at 600 r.p.m. The precipitated lipoprotein sedimented at the bottom. The supernatant liquid was decanted and precipitate was dissolved in 10% sodium bicarbonate solution. The manganese associated with lipoproteins as bicarbonate salt was removed by concentration. 5 ml of Tris-HCl buffer was added to the clear yellow supernatant and lipoproteins were completely precipitated by the addition of 2ml $MgCl_2$. The ppt was separated by centrifugation and redissolved in 5% NaCl. In order to remove contaminating serum protein, the lipoproteins were precipitated by adding 5ml Tris-HCl buffer and 2ml $MgCl_2$. The solution was then dialysed for 24 hrs. against the tris-HCl buffer to remove heparin. The dialysis bag was transferred to another flask containing 5% $BaCl_2$ solution. After 24 hrs. the insoluble Heparin-Barium salt was removed by centrifugation.

The supernatant was dialysed against Tris-HCl buffer in order to remove $BaCl_2$.This resulted in a clear yellow solution of concentrated lipoprotein. The lipoprotein isolated in this way was the mixture of LDL and VLDL which were separated by ultra centrifugation. After 24 hr. at 1,00,00 r.p.m. the VLDL formed an opalescent band at the top of the tube and the clear yellow LDL sedimented at the bottom.

ii) Isolation of HDL by precipitation with sodium Phosphatungstate and $MgCl_2$:

To 5 ml of serum were added 5ml of 4% sodium phosphotungstate. (NaPhT) and 2 ml $MgCl_2$. The ppt of LDL and VLDL were removed by centrifugation. 4.5 ml of NaPhT was added to the clear supernatant (I). The ppt which appeared immediately was free of lipids and content mostly V-globulins which was removed by centrifugation. To supernatant (II) was added 0.0875 ml of 2ml $MgCl_2$. The precipitation was completed after 2hrs. and the mixture was centrifuged for 30 minutes at 20,000 r.p.m. The clear supernatant (III) (pH 7.1) was decanted and the ppt was dissolved in 2.5 ml of solution of the following composition (1% NaCl + 0.4%, NaPhT + 0.1 M $MgCl_2$). After washing ppt was recovered by centrifugation and was suspended in 1% NaCl, and 10% Na_2CO_3 solution was added dropwise with stirring until redissolution was achieved. This concentrated neutral solution of HDL, contaminated by small amount of serum protein was further purified by ultra centrifugation.

The serum concentration of the VLDL, LDL and HDL were determined by measuring their proteins and total lipid concentrations.

iii) Protein : The protein contents were estimated by Lowry et al. method[11] and values were expressed as mg/100ml of serum.

iv) Total lipids : They were estimated gravimetrically by Folch et al. method.[12]

v) Lipoproteirs :

A suiTable aliquot of the isolated fraction was estimated according to Folch et al.[12] method by using chloroform-methanol (2:1) mixture. These extracts were evaporated and taken in a known valume of chloroform and stored in sealed stopper tubes at 20^0C. until required for further estimations.

Observations and Results:

Serum VLDL, LDL and HDL

It is evident from the Tables 1 to 3 and figures 1_f to 3_f the level of serum lipoproteins was altered in animal treated with cythion than controls. There was small but significant decrease in the concentration of serum total lipids which was largely or solely due to a reduction of the total lipid concentration in the VLDL, LDL and HDL.

Tables 1 to 3 and figures 1 *f* to 3 *f* showed total lipid changes in serum VLDL,LDL and HDL.

Table 1: Serum total lipid changes in very low density lipoprotein (VLDL) on exposure to cythion,plant extrac and analogue of phyllanthin (2b) in *albino rat*.

Weeks	Control	Induced	Plant extract	Analogue
2	61.35 ± 6.25	59.60 ± 5.50	59.82 ± 5.49	59.80 ± 5.39
4	60.95 ± 6.35	58.90 ± 5.80	59.15 ± 5.85	59.35 ± 5.37
6	60.60 ± 6.20	58.65 ± 5.00	58.75 ± 5.33	58.79 ± 5.22
8	61.25 ± 6.35	57.55 ± 5.25	57.90 ± 5.29	57.99 ± 5.32
10	60.90 ± 6.26	57.33 ± 5.4	57.85 ± 5.10	57.90 ± 5.21

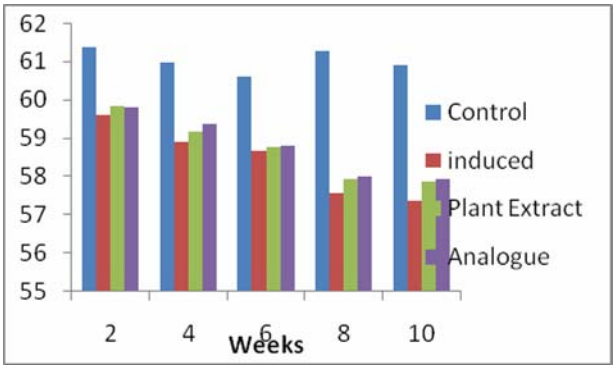


Fig. 1 *f* : Serum total lipids : *Albino rat.*

Table 2: Serum total lipid changes in low density lipoprotein (LDL) on exposure to cythion,plant extract and analogue of phyllanthin (2b) in *albino rat.*

Weeks	Control	Induced	Plant Extract	Analogue
2	71.80 ± 10.37	63.27 ± 10.45	64.11 ± 10.39	64.80 ± 10.56
4	70.85 ± 10.69	53.02 ± 10.15	54.25 ± 10.35	54.85 ± 10.38
6	70.95 ± 10.27	41.25 ± 10.90	42.65 ± 10.25	43.85 ± 10.01
8	71.10 ± 10.61	39.05 ± 10.05	40.25 ± 10.05	41.39 ± 10.22
10	71.05 ± 10.26	30.25 ± 10.4	30.75 ± 10.21	31.85 ± 10.11

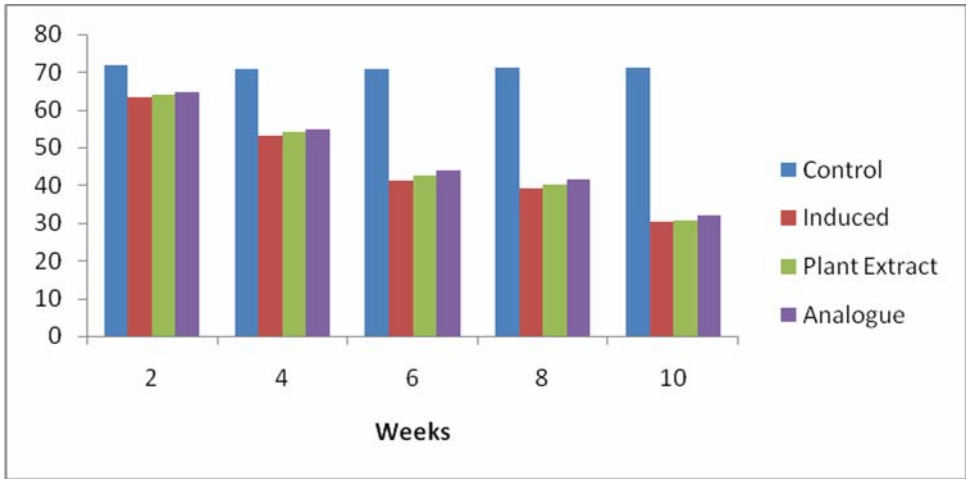


Fig. 2*f* : Serum protein : *Albino rat.*

Table No. 3 : Serum total lipid changes in high density lipoprotein (HDL) on exposure to cythion,plant extract and analogue of phyllanthin (2b) in *albino rat.*

Weeks	Control	Induced	Plant Extract	Analogue
2	113.21 ± 3.00	111.16 ± 7.6	111.79 ± 9.1	112.65 ± 2.5
4	113.22 ± 3.22	110.01 ± 7.23	111.29 ± 9.02	112.10 ± 2.56
6	113.10 ± 3.21	109.00 ± 7.33	110.90 ± 9.10	112.00 ± 2.29
8	121.97 ± 2.99	106.29 ± 6.75	109.29 ± 8.99	111.21 ± 2.10
10	113.29 ± 2.55	105.01 ± 6.59	30.75 ± 8.65	31.85 ± 2.22

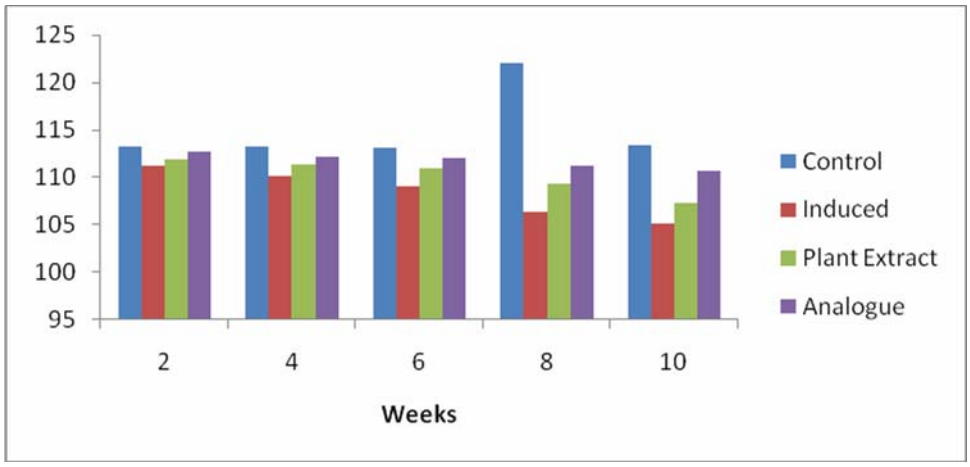


Fig. 3 *f* : Serum lipoprotein : *Albino rat*

Result

In cyhtinon treated rats there was a significant decrease in the total lipid concentration i.e.VLDL,LDL and HDL. Whereas a remarkable rie in HDL level is observed in blood serum as compared to that of control group rats.The plant extract and newly synthesised analogues under examination showed significant increase in the levels of VLDL,LDL and HDL.The study also reveled that the newly synthesised analogues have slight curative age over the crude plant extract in HDL level as compared that of VLDL and LDL.

Conclusion

The results of serum estimations in VLDL, LDL and HDL containing total lipids reveals that there is a declining trend in the values of these constituents in cythion treated *albino rats*. And the administration of phyllanthin and analogue of phyllanthin showed remarkable curative effect.

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