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Phytochemical and Pharmacological Standardisation of Polyherbal Tablets For Hepatoprotective Activity Against Carbon Tetrachloride Induced Hepatotoxicity

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ABSTRACT
Literature survey revealed that phyllanthin and hypophyllanthin from *Phyllanthus niruri*, wedenolactone from *Eclipta alba,* and kutkin from *Picrorhiza kurroa* are responsible for hepatoprotective activity, and phenolic and flavonoids are responsible for antioxidant activity. A selected polyherbal formulation composed of 7 herbal extract mixtures such as Phyllanthus niruri, *Eclipta alba, Cichorium intybus, Boerhaavia diffusa, Embelia ribes, Berberis aristata and Picrorhiza kurroa*. The phytochemical evaluation was carried out by estimation of total phenolic content and total flavonoids. The antioxidant activity was compared with ascorbic acid (ASC) and Rutin as standard. The hepatoprotective activity in carbon tetrachloride induced hepatotoxicity were studied. Assessment of liver function was made by estimating the activities of SGOT, SGPT, ALP, Cholesterol, Bilirubin and Total protein. From the study it is seen that formulation exhibit significant activity.

Keywords: Polyherbal, hepatotoxicity, hepatoprotective, antioxidant, biochemical parameters.

INTRODUCTION

In India, more than 81 medicinal plants are used in different combination in the preparation of 33-patented herbal formulations. Different polyherbal formulations are used in liver disorders to improve hepatic functioning or to correct the hepatic damage. Literature survey revealed that, S. Prabhu Nair (2006) studied protective effect of Tefroli- a polyherbal mixture (tonic) on cadmium chloride induced hepatotoxic rats. They had done the analysis of serum bilirubin and assay of marker enzymes such as transaminases and phosphatases of both serum and liver. [1] The difficulty in the acceptance of the Ayurvedic formulation or polyherbal formulation is the lack of standard quality control profiles. The quality of herbal medicine i.e. the profile of constituents in the final product has implication in efficacy and safety. Quality evaluation of plant materials and herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. Now a day's most of the ayurvedic formulations are lacking in defined quality control parameters. FDA has made the quality control and GMP mandatory for ayurvedic formulation, which has been implemented from 1st January 2003. [2] In the light of the above, present study was undertaken to evaluate the hepatoprotective effect of polyherbal tablet.

A selected polyherbal formulation composed of 7 herbal extract mixtures such as *Phyllanthus niruri*, *Eclipta alba, Cichorium intybus, Boerhaavia diffusa, Embelia ribes, Berberis aristata and Picrorhiza kurroa*.

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MATERIAL AND METHODS

The Polyherbal tablets were procured from local market of Wardha, Maharashtra, India. Folin-Ciocalteu reagent, Diagnostic Kit of biochemical parameters, Ascorbic acid, Rutin Carbon tetrachloride and other required chemicals were procured from Loba chemicals. Albino rats were used for hepatoprotective study, with prior approval from the Institutional Animal Ethical Committee (Registration No. 535/ 02/a/CPCSEA /Jan2002) of Institute of Pharmaceutical Education & Research, Wardha. Semi-autoanalyser (MERCK Microlab-300) was used as Instrument for parameter testing.

Estimation of Total phenolic content

The content of total phenolic compounds in tablets was determined by Folin-Ciocalteu reagent. A ground sample of 0.5 g of tablet powder was weighed and phenolic content were extracted with 50 ml of 80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 ml) of the extract

was centrifuged for 5 min. at 14000 rpm. An aliquot of 1 ml of 10-100µg/ml ethanolic gallic acid solution was added to 9 ml of distilled water in a 25 ml volumetric flask. A reagent blank was prepared using 10 ml distilled water and 1 ml of Folin-Ciocalteu's phenol reagent was added to it, shaken vigorously. After 5 min, 10 ml 7.5% w/v sodium carbonate solution was added. Then volume was made up to the mark with distilled water. The absorption was read after 90 minute, at room temperature at 750 nm on spectrophotometer, and calibration curve was drawn. [3] Total content of phenolic compounds in tablets were calculated by from graph.

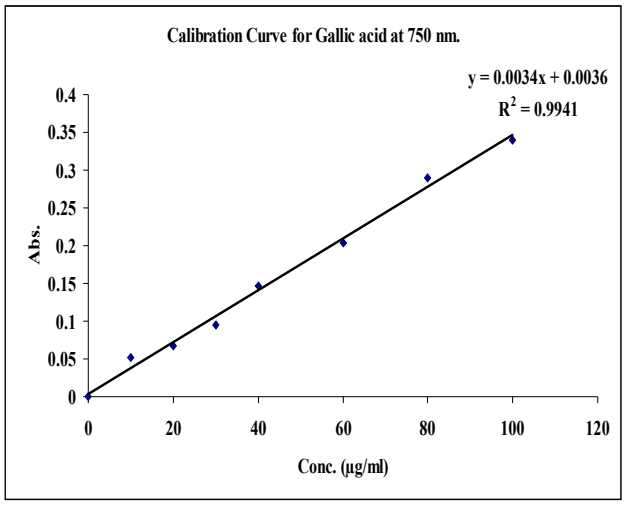


Fig 1: Standard calibration curve of Gallic acid.

Table 1: Result of Total phenolic content (%)

S. No.	Absorbance	Total phenolic content (%)	Mean*
1.	0.738	21.6	
2.	0.729	21.33	21.472 ±0.1372
3.	0.734	21.48	

Estimation of Total Flavonoid Content

It was performed by Aluminium trichloride colorimetric method. [3] A ground sample of 0.5 g of tablet powder was weighed and total flavonoid content was extracted with 50 ml of 80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 ml) of the extract was centrifuged for 5 min. at 14000 rpm. 1 ml of aliquot was mixed with 2ml aluminum trichloride in methanol (2% w/v) (Probe solution). Blank were prepared from 1ml of standard solution and diluted to 25 ml with methanolic acetic acid (0.5% v/v). The absorbance of Probe solution against standard solution was measured at 420 nm after 30 min. All the determination is carried out in triplicate. The result were expressed as Total (%) flavonoid content (TFC) in Polyherbal as Quercetin equivalent, was calculated by following formula

$$TFC\,(\%) = \frac{\text{Abs. x Dilution Factor x 100}}{\text{E 1\%, 1cm x Weight of sample (gm)}}$$

E 1%, 1cm = Specific absorption of the Quercetin $ALCl_3$ Complex (500)

Table 2: Result of Total Flavonoids content (%) of tablets

S. No.	Absorbance	Total Flavonoids (%) of tablets	Mean ± SD
1.	0.811	40.55	
2.	0.803	40.15	40.20±0.3279
3.	0.798	39.9	

Antioxidant activity

DPPH Radical scavenging activity

The free radical scavenging activity of was measured *in vitro* by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay. About 0.1 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of drug dissolved in ethanol at different concentrations (10-100µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC_{50} value of the drug was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. [4] The capability to scavenge the DPPH radicals was calculated using the following formula,

$$\text{DPPH scavenged (\%)} = \frac{\text{(A cont - A test)}}{\text{A cont}} \times 100$$

Where,

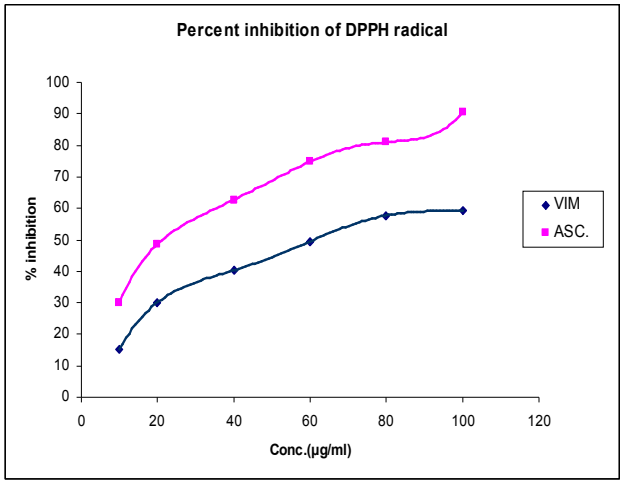
A_{cont} is the absorbance of the control reaction mixture.

A_{test} is the absorbance of sample at different concentrations.

Table 3: Percentage inhibition of DPPH radical and IC_{50} values of tablets

S. No.	Concentration (µg/ml)	% Inhibition*	
		Sample	ASC
1.	10	15.2 ±0.200	30.18 ±1.21
2.	20	30.15 ±0.030	48.64 ±0.60
3.	40	40.31 ±0.060	62.69 ±1.67
4.	60	49.5 ±0.412	74.78 ±1.65
5.	80	57.75 ±0.060	81.00 ±1.67
6.	100	59.23 ±0.098	90.56 ±1.66
	IC_{50} Values	32.89	16.6

*represents Mean ±S.D. n=3



VIM- Polyherbal tablet
ASC.- Ascorbic acid

Fig 2: DPPH scavenging activity of polyherbal tablet

Nitric oxide scavenging activity

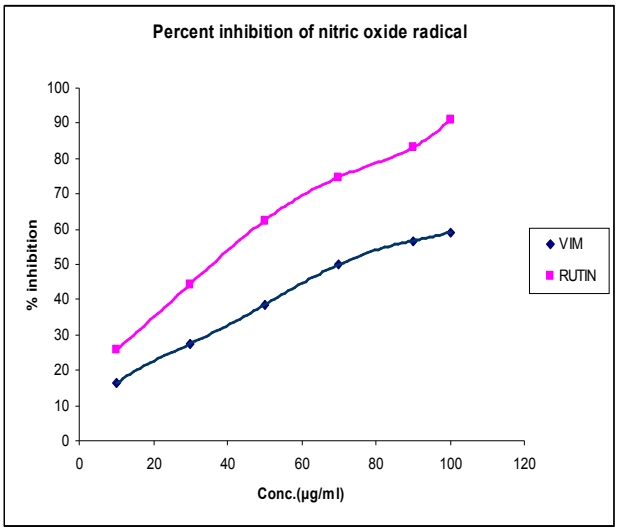
The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and extract or standard solution (0.5 ml) was incubated at 25ºC for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthylethylene diamine dihydrochloride (1%) was added to it, mixed and allowed to stand for 30 min. A pink colored chromophore was formed in diffused light.

The absorbance of these solutions was measured at 547 nm against the corresponding blank solutions. The IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical. [5] All determinations were performed in triplicates.

Table 4: Percentage inhibition of nitric oxide radical and respective IC_{50} values

S. No.	Concentration (µg/ml)	% Inhibition*	
		Sample	Rutin
1.	10	16.24 ±0.0436	25.86 ±1.27
2.	30	27.51 ±0.03	44.45 ±0.60
3.	50	38.55 ±0.1308	62.40 ±1.47
4.	70	49.98 ±0.1249	74.70 ±0.60
5.	90	56.75 ±0.03	83.16 ±1.80
6.	100	59.12 ±0.04	91.10 ±1.86
.	IC_{50} Values	30.78	18.38

*represents Mean ±S.D., n=3



VIM- Polyherbal tablet
RUTIN- Standard
Fig 3: Nitric oxide radical scavenging activity of Polyherbal tablets

Hepatoprotective activity

The hepatoprotective study was conducted on albino rats (150-250 g). They were kept at standard animal housing conditions (Temperature 23 ± 1°C and relative humidity 55 ± 10%) and 12 h light/ dark cycle. The animals were maintained on standard diet in large specious polypropylene cages and supplied with water *ad libitum*. They were used for studies after an acclimatization period of 10 days in laboratory environment activity. [6] Albino rats of either sex were divided into following group with six animals in each group.

Group I : Normal, received normal rat fed & water
Group II : Control, CCl4 intoxicated (0.7ml/kg by intraperitoneal injection)
Group III : Standard drug treated, (Silymarin, 100 mg/kg, orally)
Group IV : Polyherbal treated (150 mg/kg, orally)
Group V : Polyherbal treated (300 mg/kg, orally)

The Polyherbal tablets were administered to the experimental rats in two doses i.e. 150 mg/kg, p. o. and 300 mg/kg p. o. by dispersing it in 1% Tween 80.
The method consists of three steps:

1. Normal levels of serum Glutamate Pyruvate Transaminase (SGPT) and serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total protein, Bilirubin and Cholesterol were determined by withdrawing blood samples directly by puncturing the Retro-orbital plexus on the first day of study. Collected blood was centrifuged at 2500 rpm for separation of serum. Serum was analyzed on semi-autoanalyser for these parameters.
2. To the animals 0.7ml per kg body weight of carbon tetrachloride (CCL_4) was administered intraperitoneally for five days. On the sixth day enzymatic levels were noted.
3. After intoxication with CCL_4, Polyherbal and Standard Silymarin were administered for five days. On the 11th day the serum levels were recorded. For determination of significant intergroup difference each parameter was analyzed separately.

RESULTS AND DISCUSSION

Total phenolic compounds (mg/gm) of the tablet were found to be 21.472 ±0.1372 (Table 1). The total flavonoid content of the tablet was found to be 40.20 ±0.3279 (Table 2). The antioxidant activity when compared with ascorbic acid (ASC) as standard, the IC_{50} value of tablet was found to be 32.89 (Table 3). And when compared with rutin as standard, the IC_{50} value of tablet was found to be 30.78 (Table 4). Assessment of liver function was made by estimating the activities of SGOT, SGPT, ALP, Cholesterol, Bilirubin and Total protein. SGPT and SGOT are the enzymes originally present in higher concentration in cytoplasm. The Polyherbal tablet had shown significant decrease in enzyme level of SGOT, SGPT, ALP, Cholesterol, Bilirubin and significant increase in enzyme level of Total protein ($P< 0.01$) (Table 5, 6).

Table 5: Effect of Polyherbal tablets on Serum SGOT Level, Serum SGPT Level and Serum Alkaline Phosphatase Level in hepatotoxic rats

S. No.	Groups	Serum SGOT [mg/dl]	Serum SGPT [mg/dl]	Serum Alkaline Phosphatase [U/l]
		[Mean ± SD]	[Mean ± SD]	[Mean ± SD]
1.	Normal [GR.I]	188.91 ±1.774	88.75 ±1.293	171.16 ±0.947
2.	Control [GR.II]	337.26 ±1.885*	134.62±0.801*	505.45 ±2.589*
3.	Hepatotoxic +Standard [GR.III]	217.18 ±2.348**	98.69 ±1.043**	221.59 ±2.344**
4.	Hepatotoxic+Polyherbal tablet (150mg/kg) [GR.IV]	245.87 ±1.982**	103.64 ±1.65**	248.73 ±1.281**
5.	Hepatotoxic+Polyherbal tablet (300mg/kg) [GR.V]	211.72 ±1.741**	94.51 ±0.796**	226.43 ±1.428**

Values are given as Mean ± SD of six rats in each group.
Control was compared with the normal, $p < 0.01$*
Experimental groups were compared with the control, $p < 0.01$**

Table 6: Effect of Polyherbal tablets on Serum Total Protein Level, Serum Cholesterol Level and Serum Bilirubin Level in hepatotoxic rats

S. No.	Groups	Serum Total Protein [U/l] [Mean± SD]	Serum Cholesterol [mg/ml] [Mean ± SD]	Serum Bilirubin [mg/dl] [Mean ± SD]
1.	Normal [GR.I]	7.11 ±0.45	68.97 ±0.961	0.78 ±0.021
2.	Control [GR.II]	5.15 ±0.049*	119.50 ±1.436*	2.388 ±0.017*
3.	Hepatotoxic +Standard [GR.III]	6.58 ±0.028**	92.51 ±1.60**	0.916 ±0.019**
4.	Hepatotoxic+Polyherbal tablet(150mg/kg) [GR.IV]	6.42 ±0.028**	100.54 ±2.022**	1.21 ±0.016**
5.	Hepatotoxic+Polyherbal tablet(300mg/kg) [GR.V]	6.24 ±0.021**	87.74 ±1.915**	1.04 ±0.017**

Values are given as Mean ± SD of six rats in each group.
Control was compared with the normal, $p < 0.01$*
Experimental groups were compared with the control, $p < 0.01$**

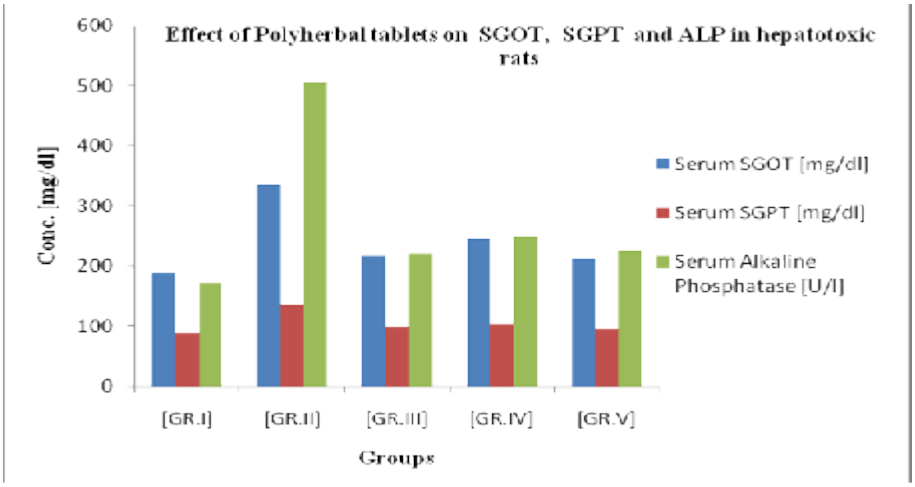


Fig. 4: Effect of Polyherbal tablets on Serum SGOT Level, Serum SGPT Level and Serum Alkaline Phosphatase Level in hepatotoxic rats

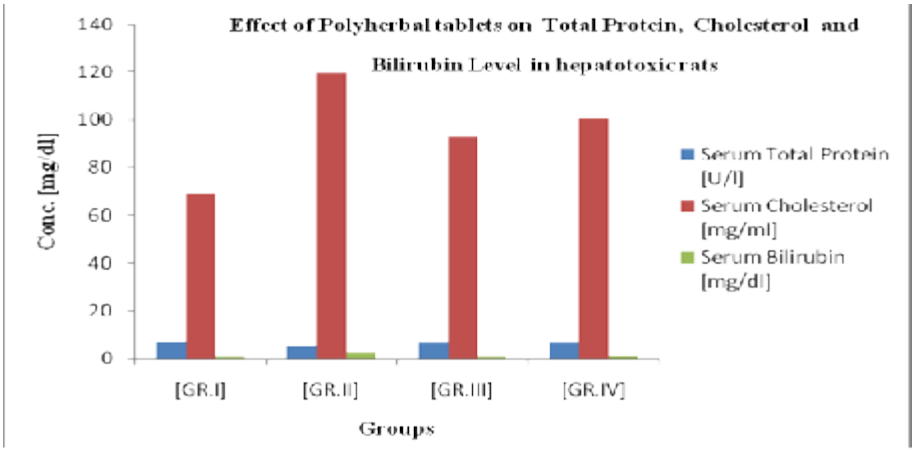


Fig 5: Effect of Polyherbal tablets on Serum Total Protein Level, Serum Cholesterol Level and Serum Bilirubin Level in hepatotoxic rats

From the results of Phytochemical screening it was concluded that the Phytochemical present in tablets were responsible for antioxidant activity and the determination of phenolic, flavonoids content gives its quantitative values. The results of estimation of the antioxidant activity of polyherbal tablets prove its action on free radicals. The polyherbal tablets were found to be effective against CCl_4 induced hepatic damage in rats, by reversal of increased serum level of SGOT, SGPT, ALP, Cholesterol, Bilirubin and decreases level of Total Protein which occurs during hepatotoxicity.

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