



HEPATOPROTECTIVE ACTIVITY OF LIVERGEN, A POLYHERBAL FORMULATION AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Polyherbal formulations available with a wide range of indications like protective to liver, appetite and growth promoters, gastrointestinal and hepatic regulator, as treatment for hepatic dysfunction, for hepatic regeneration as well as liver stimulant and tonic. Despite the widespread use, there is a lack of scientific evidence on their efficacy and safety. Literature survey revealed that Andrographolide from *Andrographis paniculata*, wedenolactone from *Eclipta alba*, and kutkin from *Picrorrhiza kurroa* are responsible for hepatoprotective activity, and phenolic and flavonoids are responsible for antioxidant activity. A selected polyherbal formulation composed of various herbal extract mixtures such as *Andrographis paniculata*, *Apium graveolens*, *Berberis lycium*, *Carum copticum*, *Cichorium intybus*, *Cyperus rotundus*, *Eclipta alba*, *Ipomoea turpethum*, *Oldenlandia corymbosa*, *Picrorrhiza kurroa*, *Plumbago zeylanica*, *Solanum nigrum*, *Tephrosia purpurea*, *Terminalia arjuna*, *Terminalia chebula*, *Trigonella foenumgraecum*. 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

About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations¹. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy². Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated³.

In the present study, a polyherbal hepatoprotective formulation, namely Livergen was selected from 40 such formulations. The criteria for selection was based on (i) claimed as Ayurvedic medicine, (ii) commercially available, (iii) liquid formulations for easy administration, (iv) with known hepatoprotective activity of one or more plants, and (v) sufficient shelf life. This herbal drug have been traditionally used for liver diseases and the polyherbal formulations, claimed to be Ayurvedic medicines are being sold as liver tonics.

Literature survey revealed that, S. Prabhu Nair (2006) studied protective effect of Tefrol- 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 liver⁴. 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 2003⁵. In the light of the above, present study was undertaken to evaluate the hepatoprotective effect of polyherbal formulation.

A selected polyherbal formulation composed of various herbal extract mixtures such as *Andrographis paniculata*, *Apium graveolens*,

Berberis lycium, *Carum copticum*, *Cichorium intybus*, *Cyperus rotundus*, *Eclipta alba*, *Ipomoea turpethum*, *Oldenlandia corymbosa*, *Picrorrhiza kurroa*, *Plumbago zeylanica*, *Solanum nigrum*, *Tephrosia purpurea*, *Terminalia arjuna*, *Terminalia chebula*, *Trigonella foenumgraecum*.

MATERIAL AND METHODS

The Polyherbal formulations 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. Silymarin was provided by Sigma labs. Albino rats were used for hepatoprotective study, with prior approval from the Institutional Animal Ethical Committee (RegistrationNo.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 formulations was determined by Folin- Ciocalteu reagent. A sample of 0.5ml was added to 1 ml of 10-100 µg/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 drawn⁶. Total content of phenolic compounds in Livergen formulations were calculated by from graph.

Estimation of total flavonoid content

It was performed by Aluminium trichloride colorimetric method⁶. 0.5 ml Livergen was extracted with 50 ml of 80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2ml) of the extract was centrifuged for 5min. at 14000 rpm. 1ml of aliquot was mixed with 2ml aluminium 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 express as Total (%) flavonoid content (TFC) in polyherbal formulation as Quercetin equivalent was calculated by following formula:

$$\text{TFC (\%)} = \frac{\text{Abs.} \times \text{Dilution Factor} \times 100}{E^{1\%}_{1\text{cm}} \times \text{Weight of sample (ml)}}$$

$E^{1\%}_{1\text{cm}}$ = Specific absorption of the Quercetin AlCl_3 Complex (500)

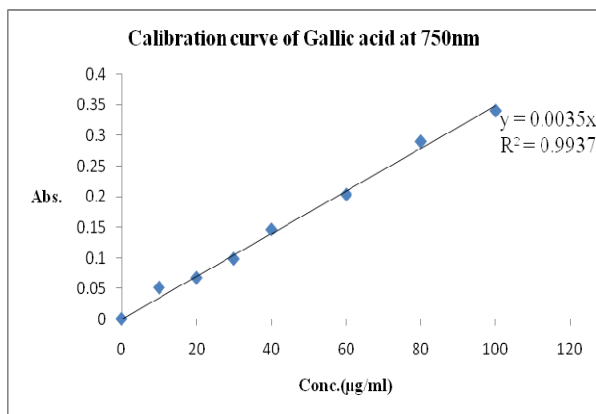


Fig. 1: Standard calibration curve of gallic acid

Antioxidant activity

DPPH Radical scavenging activity

The free radical scavenging activity of Livergen 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 Livergen dissolved in ethanol at different concentrations (10–100 µg/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 standard⁷. The capability to scavenge the DPPH radicals was calculated using the following formula,

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

Where,

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

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

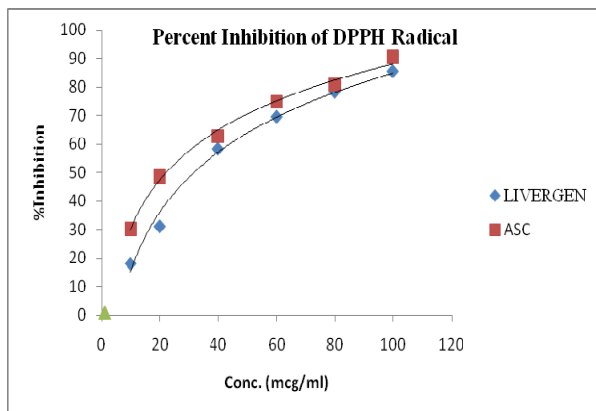


Fig. 2: DPPH scavenging activity of livergen polyherbal formulation

LIVERGEN- Polyherbal formulation

ASC - Ascorbic acid

Hepatoprotective activity

The albino rats weighing 170- 210 gm were used for the hepatoprotective study. They were kept at standard animal housing conditions (Temperature 23 ± 10 C and relative humidity $55 \pm 10\%$) and 12 hour light/ dark cycle. The animals were maintained on standard diet in large specious polypropylene cages and supplied with water *ad-libitum*. The protocol was approved by Institutional Animal Ethical Committee (RegistrationNo.535/ 02/a/CPCSEA /Jan2002)⁹.

The dose of Livergen was calculated to precisely match with the human doses employed according to the manufacturer’s instructions. The average recommended human dose of 20 ml/day was converted to that of rats by a standard conversion table¹⁰. The formulation was 10 times diluted and was given by oral route as pretreatment (2.60 ml/kg bw/day).

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, CCl_4 intoxicated (0.7ml/kg by Intraperitoneal injection)

Group III : Standard drug treated, (Silymarin, 100 mg/kg, orally)

Group IV : Livergen treated (2.60 ml/kg bw, orally)

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 , Livergen 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

The polyherbal hepatoprotective formulation, Livergen was subjected for determination of phenolic, flavonoids content. Total phenolic compounds (mg/gm) of the formulation were found to be 2126.823 ± 0.1472 (Table 1).

Table 1: Total phenolic content (%) of livergen formulation

Sr. no.	Absorbance	Total phenolic content (%)	Mean*
1	0.941	26.88	26.823±0.1472
2	0.949	27.11	
3	0.934	26.68	

The total flavonoid content of the formulation was found to be 49.00 ± 0.1279 (Table 2).

Table 2: Total flavonoids content (%) of livergen Formulation

Sr. No	Absorbance	Total flavonoids (%) of formulations	Mean ± SD
1.	0.983	49.15	49.00 ±0.1279
2.	0.973	48.65	
3.	0.984	49.2	

The antioxidant activity was compared with ascorbic acid (ASC) as standard, the IC₅₀ value of formulation was found to be 62.45 (Table 3). The results of estimation of the antioxidant activity of polyherbal formulations prove its action on free radicals.

Table 3: Percentage inhibition of DPPH radical and IC₅₀ values of livergen formulation

Sr. no.	Concentration(µg/ml)	% Inhibition*	
		Sample	ASC
1.	10	18.12 ±0.121	30.18 ±1.21
2.	20	31.15 ±0.102	48.64 ±0.60
3.	40	58.31 ±0.098	62.69 ±1.67
4.	60	69.51 ±0.143	74.78 ±1.65
5.	80	78.25 ±0.78	81.00 ±1.67
6.	100	85.44 ±0.094	90.56 ±1.66
	IC ₅₀ Values	62.45	66.6

*represents Mean ±S.D n=3

Liver damage and recovery from damage was assessed on eleventh day by measuring serum marker enzymes, biochemical changes in liver Injury produced by CCl₄ seems to be mediated by a reactive metabolite, trichloromethyl free radical (·CCl₃) formed by the hemolytic cleavage of CCl₄ or by an even more reactive species, trichloromethylperoxy free radical (CCl₃COO·) formed by the reaction of ·CCl₃ with O₂. This biotransformation is catalyzed by a cytochrome P₄₅₀-dependent monooxygenase. The toxicity produced by CCl₄ is thought to be due to the reaction of free radicals (·CCl₃ or CCl₃COO·) with lipids and proteins. Assessment of liver function was made by estimating the activities of SGOT, SGPT, ALP, Cholesterol, Bilirubin and Total protein. The Polyherbal formulation Livergen 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 4,5). The polyherbal hepatoprotective formulation Livergen was effective at normal dose used in this study justifying its use as a hepatoprotective agent.

Table 4: Effect of Livergen Formulation On Serum SGOT Level, Serum SGPT Level And Serum Alkaline Phosphatase Level In Hepatotoxic Rats

Sr. No.	Groups	SerumSGOT [mg/dl]	SerumSGPT [mg/dl]	Serum Alkaline Phosphatase [U/l]
		[Mean ± SD]	[Mean ± SD]	[Mean ± SD]
1.	Normal [GR.I]	178.91 ±1.667	108.47 ±1.963	175.85 ±1.567
2.	Control [GR.II]	364.24 ±1.857*	213.68 ±1.141*	506.55 ±1.849*
3.	Hepatotoxic +Standard [GR.III]			
4.	Hepatotoxic+Livergen (2.6ml/kg) [GR.IV]	197.2 ±1.527**	116.32±1.483**	223.82 ±1.547**
		195.42 ±1.895**	127.35 ±1.972**	227.88 ±1.742**

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**

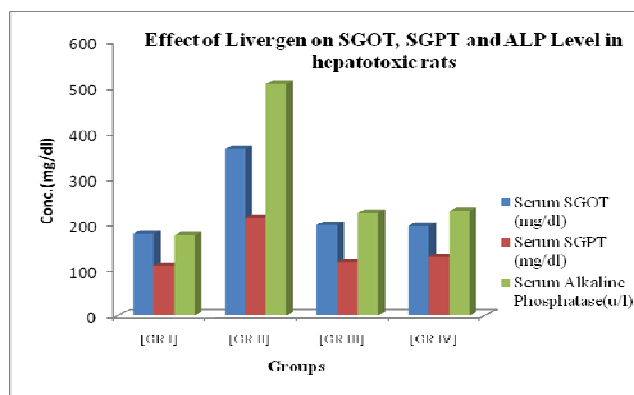


Fig. 3: Effect of Livergen on Serum SGOT Level, Serum SGPT Level and Serum Alkaline Phosphatase Level in hepatotoxic rats

Table 5: Effect of polyherbal formulation on serum total protein level, serum cholesterol level and serum bilirubin level in hepatotoxic rats

Sr. No.	Groups	Serum Total Protein [U/l]	Serum Cholesterol [mg/ml]	Serum Bilirubin [mg/dl]
		[Mean ± SD]	[Mean ± SD]	[Mean ± SD]
1.	Normal [GR.I]	7.09 ±0.158	75.76 ±1.263	0.89 ±0.113
2.	Control [GR.II]	5.67 ±0.198*	156.58 ±1.928*	2.768 ±0.627*
3.	Hepatotoxic +Standard [GR.III]			
4.	Hepatotoxic+Livergen (2.6ml/kg) [GR.IV]	6.04 ±0.149**	87.59 ±1.235**	1.092 ±0.187**
		6.12 ±0.453**	98.28 ±1.123**	1.382 ±0.272**

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**

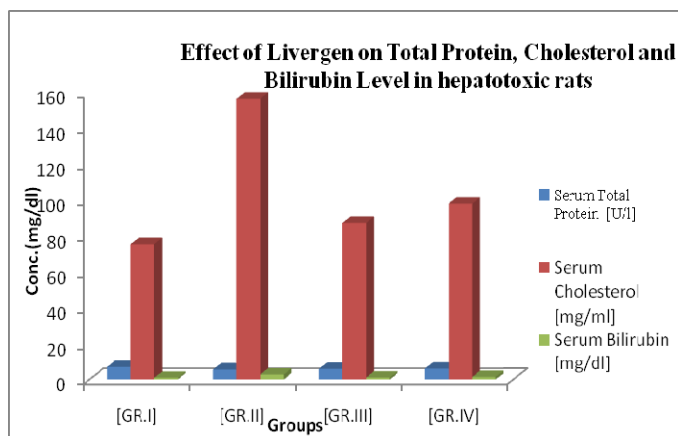


Fig. 4: Effect of Livergen on Serum Total Protein Level, Serum Cholesterol Level and Serum Bilirubin Level in hepatotoxic rats

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