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K. Dinesh
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

V. Sivakumar
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

B. Selvapriya
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

E. Deepika
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

A. Mohamed Sadiq
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

Correspondence:
V. Sivakumar
P. G. and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India.

Evaluation of hepatoprotective polyherbal formulations contains some Indian medicinal plants

K. Dinesh, V. Sivakumar, B. Selvapriya, E. Deepika, A. Mohamed Sadiq

Abstract
The aim of the present study was to evaluate the hepatoprotective effect of poly herbal formulations contains some Indian medicinal plant on paracetamol induced hepatic damaged experimental animals.
Materials and methods: Paracetamol (3 g/kg, p.o) was administered a single dose to induce hepatotoxicity. F-I, F-II and F-III (200 mg/kg, p.o) and silymarin (25 mg/kg, P.o) were administered once daily for 8 days. The degree of hepatoprotection was measured using morphological parameters of changing in colour and weight of liver are determined. Biochemical parameters in serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), bilirubin and plasma protein. The histopathological parameters of histological changes in the liver architecture like architecture of hepatic lobules, swelling of liver cell, fatty changes, focal necrosis, inflammatory cell infiltration around portal areas, kupffer cell hyperplasia etc. and the functional parameters of pentobarbitone sleeping time was used as a functional parameter.
Results: F-I, F-II and F-III pretreatment showed normal morphological parameters signs and significant effect of serum enzymes, total protein and bilirubin levels. Highly significantly protect the hepatic damage and morphological and histopathological changes and less pentobarbitone sleeping time were observed in all the three poly herbal formulations treated rats.
Conclusion: The result of this study strongly indicated that the entire three poly herbal formulations have got a hepatoprotective action against paracetamol induced hepatic damage in experimental animals.

Keywords: Poly herbal formulation, hepatoprotective, paracetamol, silymarin, histopathology.

1. Introduction
The liver is a vital organ having a wide range of functions including detoxification, protein synthesis and production of biochemical necessary for digestion. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Liver is the key organ to maintenance, performance and regulating homeostasis in the body. But liver is continuously and variedly exposed to exogenous substances like environmental toxins, drugs and alcohol which can eventually lead to various liver disorders, generally presenting as a distinct patterns of diseases such as hepatocellular, cholestatic (obstructive), or mixed type of liver disorders [2].
Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today [3]. Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders because of high cost and additional adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety.
In the absence of reliable modern hepatoprotective drugs, in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. The different plants in the herbal mixture will have different modes of action for curing the disease and in the combined form may sometimes exhibit synergistic activity (enhanced activity than of the individual herbs). Components of the plants, which are not active themselves, can act to improve the stability, solubility and bioavailability or half-life of the active compounds. Hence a particular active principle in the pure form may have only a fraction of the pharmacological activity that it has in its plant matrix, which again highlights the importance in using the plant as a whole or a mixture of plants for treating a diseases.

A greater deal of research has been carried out to evaluated scientific basis for the claimed hepatoprotactive activity of herbal agents as single agent or in formulation. The plant herbal formulations F - I, F- II and F- III under study contain plant ingredients like hydroalcoholic extract of *Tinospora cordifolia* and ethanol extract of *Boerhavia diffusa*, *Phyllanthus amaraus*, *Euphorbia hirta*, *Wedelia chinensis.* The form of extract whether hydroalchol or ethanol and contact in dose are based on the traditional knowledge and reports present on these plants.

Formulations are developed based on Ayurvedic principles where plants are included for antioxidant activity, hepatoprotactive activity, bio availability enhancement and specific activity in modulation of different liver disease conditions as many of these herbal ingredients are known to have liver modifying activity. *Tinospora cordifolia* is reported to antioxidant and hypoglycemic [4], *Boerhavia diffuse* is known for its antioxidant activity [5], decoction of the *Phyllanthus amaraus* is known traditionally for its effect in jaundice [6], *Euphorbia hirta* is found to be protective in paracetamol induced liver damage [7], *Wedelia chinensis* is reported to hepatoprotactive activity [8, 9]. We have undertaken this study to evaluate the efficacy of these formulations in experimental animals in which acute hepatotoxicity was induced by paracetamol.

2. Materials and methods

2.1 Plants collection and Preparation of plant extract

Fresh and matured medicinal plants were collected from Adhiparasakthi Agricultural College campus Medicinal garden in kalavai, India. All the plant materials was identified and authenticated in the plant anatomy research centre, Chennai. The plants voucher specimen number: PARC/2013/2026-2030 was deposited in the center herbarium.

All the medicinal plants and plants part were subjected to surface sterilization using ethanol and then dried in shade. The all the dried plants was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh). All the medicinal plant powdered samples (100 g) were defatted by treating with pet-ether and then extracted with ethanol and hydro alcoholic solvent by using soxhlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4 ºC. From this stock, fresh preparation was made whenever required.

2.2 Preliminary phytochemical analysis

All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents [10].

2.3 Animals

The healthy adult male *wistar albino* rats (weighing 180 – 210 g) were used in the experiments. Animals were housed in polypropylene cages at 22±2 ºC with relative humidity of 45-55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan Lever Ltd., India) and water *ad libitum*. Ethical clearance was obtained from the Institutional Animal Ethical Committee, CPCSEA, India (Reg. No. 282/ac/09/CPCSEA).

2.4 Acute toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operative and development guidelines No. 423 [11]. *Wistar albino* rats of either sex were divided into six groups with six animals each. Formulations - III was administered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, and 2000 mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days.

2.5 Experimental protocol

The rats were divided into six groups with six in each group. Group-I Animal were administrated a single daily dose of carboxymethyl cellulose (1 ml of 1% w/v, p.o body weight) served as healthy control. Group -II animals served as paracetamol control, which received paracetamol (3 g/kg) as a single dose on day 8. Group-III animals received Formulation - I (200 mg/kg, p.o) once daily for 8 day. Group – IV animals received Formulation - II (200 mg/kg, p.o) once daily for 8 days. Group – V animals received Formulation – III (200 mg/kg, p.o) once daily for 8 days. Group – VI animals served as reference control and received standard drug silymarin using for treatment of liver damages (25 mg/kg, p.o) once daily for 8 days.

All groups except group I received paracetamol (3 g/kg) as a single dose after 30 min. of 8 day treatment of the herbal during. All the test drugs and paracetamol were administered orally by suspending in 0.5% CMC solution. Animals were sacrificed by cervical dislocation, 48h after the last dose of the drug. The liver samples were dissected and blood was collected.

2.6 Histopathological studies

For histological studies, the liver tissues were fixed with 10% phosphate buffer neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin, thin sections (5 µm) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue [12].

2.7 Pentobarbitone sleeping time

At the last day of treatment, Pentobarbitone sodium in water for injection (35 mg/kg.b.w) was administrated intraperitoneally to all groups (I-VI). Food was withdrawn and water gives *ad libitum* 12 h before treatment. All the experiments were conducted between 09.00 am to 05.00 pm in temperature controlled room. The animals were placed on table after loss of righting reflex. The time interval between loss and regain of righting reflex was measured as Pentobarbitone Sleeping Time (PST). This functional parameter was used to determine the metabolic activity of the liver.

2.8 Statistical analysis

The statistical analysis was performed by ANOVA under one way classification followed by Bonferroni multiple comparison test, changes were considered significant at the P-value of < 0.05 and < 0.01 level of significance. The values were expressed as mean ± SD.

2.9 Assessment of Hepatoprotective activity

The hepatoprotective activity was assessed by using four parameters viz, morphological, biochemical, histopathological and function parameters.

1. Morphological parameters: Changing in colour and weight of liver are determined.
2. Biochemical parameters (Liver function test): blood samples collected were examined for changes in serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), bilirubin and plasma protein.
3. Histopathological parameters: this include the histological changes in the liver architecture like architecture of hepatic lobules, swelling of liver cell, fatty changes, focal necrosis, inflammatory cell infiltration around portal areas, kupffer cell hyperplasia etc.
4. Functional parameters: Pentobarbitone Sleeping Time was used as a functional parameter for ensuring the liver damage or effect of test formulations.

3. Result and discussion

Paracetamol (N-acetyl-p-amino phenol, acetaminophen) a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and human at high doses. It is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates. However, hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites. Over dose of paracetamol leads to mitochondrial dysfunction followed by act hepatic necrosis [13].

All these events culminate in functional and morphological changes leading to loss of integrity of cell membranes which is evidenced by the rise in levels of serum marker enzymes.

Suspension formulations were prepared using combination of various extracts of *Tinospora cordifolia, Boerhavia diffuse, Phyllanthus amaraus, Euphorbia hirta,* and *Wedelia chinensis* that showed significant hepatoprotective activity (Table 2). The suspensions were evaluated for the Preliminary phytochemical, organoleptic and hepatoprotactive activity. In table 1 shows the details of preliminary phytochemical result of the different plant extracts. The major active principal phytocompounds are present in the plant extracts. In the organoleptic studies all polyherbal formulations exhibited pleasant appearance and acceptable odor and bitter taste.

According to acute toxicity study the LD_{50} value for the formulation – III. The formulation – III administered at a dose of 2000 mg/kg through oral did not show any adverse sign and symptoms of mortality during the observation period. Based on this observation the stating dose was selected as $1/10^{th}$ (200 mg/kg) was taken in this study.

And table 3 shows the effects of liver weight and PBT sleeping time of control and paracetamol induced hepatotoxicity rats. Paracetamol in dose of 3g / kg. of b.w., produce acute hepatic damage. There is significantly increased in liver weight and PBT sleeping time in paracetamol induced rats. The pre-treated of polyherbal formulations (F-I, F-II & F-III) animals liver weight and PBT sleeping time was lower when compared to the Paracetamol treatment animals.

In table 4 exhibits the effects of suspension formulations on various biochemical parameters such as SGOT, SGPT, SALP, bilirubin and plasma protein. The paracetamol induced animals SGOT, SGPT, SALP and bilirubin concentration were increased significantly. And plasma protein was significantly decreased. The pretreatment of herbal formulations contains active compounds significantly to prevent the acute hepatotoxicity in animals because the SGOT, SGPT, SALP, bilirubin and plasma protein are maintain in near normal levels when compared to paracetamol induced groups. Formulations I, II & III were able to reduce level of enzymes, indicating that they were protective to hepatocytes and maintained normal liver physiology and further cause stabilization of plasma membrane and prevent and regeneration of liver cells. When compared to F –I and F-II, the F-III have the highly significant to prevent the paracetamol induced hepatotoxicity in experimental animals.

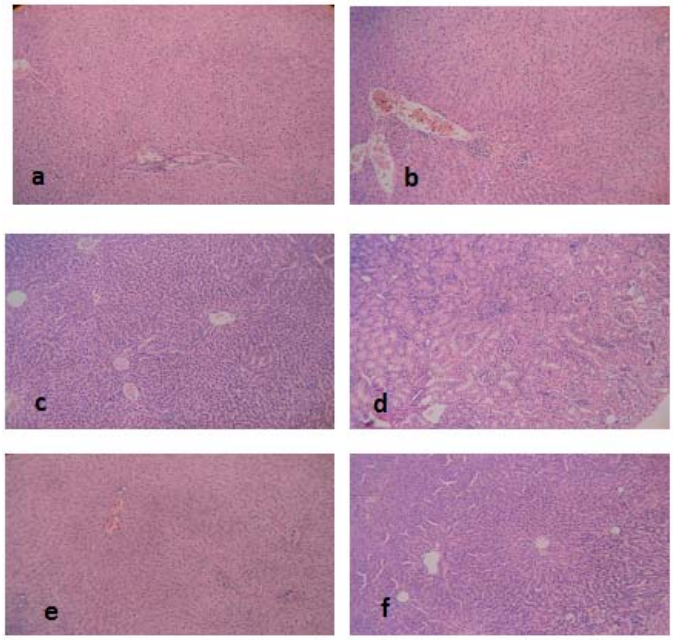

Fig 1: Histopathological of hepatic cells of (a) Normal control rat, (b) Paracetamol Toxic control (3 g/kg), (c) Paracetamol +F-I (200 mg/kg), (d) Paracetamol + F-II (200 mg/kg), (e) Paracetamol + F-III (200 mg/kg), (f) Paracetamol + Silymarin (25 mg/kg).

The histological observation basically supported the results obtained from serum enzyme assays. Liver of the normal control rats were without any pathological changes or abnormalities. Paracetamol control rats showed various degrees of pathological changes, starting from centrilobular necrosis of hepatic cells to central lobular fatty degeneration with inflammation. Section of liver taken from the rats treated with standard drug silymarin showed a hepatic architecture similar to that of normal showed a hepatic architecture similar to that normal control rats. In contrast, pre-treated of polyherbal formulations (F-I, F-II & F-III) (200 mg/kg, p.o) attenuated the pathological changes and showed significant protection against paracetamol induced hepatic damage [Figure 1].

Table 1: Phytochemical analysis of different plant extracts

Plant name	Alkaloids	Carbohydrate	Glycosides	Saponins	Phytosterol	Phenol	Flavanoids	Protein	Triterpenoids
Boerhavia diffuse (Ethanol Extract)	+	+	+	-	+	++	-	-	+
Phyllanthus amaraus (Ethanol Extract)	+	+	+	-	+	+	+	-	+
Tinospora cordifolia (Hydroalcoholic Extract)	+	+	+	+	+	++	+	-	-
Euphorbia hirta (Ethanol Extract)	+	+	+	+	+	+++	+	-	-
Wedelia chinensis (Ethanol Extract)	+	+	-	-	-	-	+	+	-

+ Present; ++ More amount present; - Absent

Table 2: Composition of poly herbal formulations

Ingredients % w/w	**F – I**	**F – II**	**F- III**
Boerhavia Diffuse (Ethanol Extract)	1.3 g	1.0 g	0.5 g
Phyllanthus amaraus (Ethanol Extract)	1.0 g	-	0.8 g
Tinospora cordifolia (Hydroalcoholic Extract)	1.28 g	-	0.9 g
Euphorbia Hirta (Ethanol Extract)	-	1.2 g	0.5 g
*Wedelia Chinensis (*Ethanol Extract)	-	1.3 g	0.8 g
Sorbitol	5.0 g	5.0 g	5.0 g
Sucrose	12 g	12 g	12 g
Carbox Methyl Cellulose (CMC)	2.0 g	2.0 g	2.0 g
Olive Oil	1 g	1 g	1 g
Distilled Water	400 ml	400 ml	400 ml

Table 3: Effect of herbal formulations in liver weight and PBT sleeping time of control and paracetamol induced hepatotoxicity rats.

Groups	Liver weight (g)	PBT sleeping time (min.)
Control	6.52± 0.1	63.31± 2.1
Paracetamol induced group	8.51± 1.1***	130.21± 1.0***
Paracetamol + 200 mg F-I	6.92± 0.2***	75.02± 2.0***
Paracetamol + 200 mg F – II	6.51± 1.1***	74.23±1.0***
Paracetamol + 200 mg F -III	6.41± 0.3***	69.30± 2.0***
Silymarin (25 mg)	6.61± 1.2***	67.11±0.1***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test,* P<0.01, ** P<0.05. PBT:** Pentobarbitone Sleeping Time

Table 4: Effect of herbal formulations in biochemical parameters of control and paracetamol induced hepatotoxicity rats.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP(K.A Units)	Bilirubin- total (mg/dl)	Plasma protein (g/dl)
Control	225.11± 0.1	110.21± 1.2	150.51± 0.3	0.51± 1.1	6.52± 0.5
Paracetamol induced group	985.11± 0.2***	950.22± 1.0***	665.62±1.1***	1.07±0.1***	3.21±1.6***
Paracetamol + 200 mg F-I	350.21± 0.1***	265.02± 2.0***	185.23±2.0***	0.82±3.1***	5.26±2.1***
Paracetamol + 200 mg F – II	300.13± 2.0***	245.232±1.0***	175.11±2.6***	0.69±2.4***	5.91±2.2***
Paracetamol + 200 mg F -III	245.20± 0.2***	197.31± 2.0***	162.01±3.4***	0.61±3.1***	6.21±1.0***
Silymarin (25 mg)	395.31± 1.0***	192.31±0.1***	165. ± 1.7***	0.62±1.2***	5.84±0.2***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test,* P<0.01, ** P<0.05. SGOT:** serum glutamate oxaloacetate transaminase**, SGPT:** serum glutamate pyruvate transaminase**, ALP:** Alkaline phosphatase.

4. Conclusion

From the above result it may be concluded that the herbal drug formulations possesses significance hepatoprotective activity in therapeutic and prophylactic mode in acute toxicity models and are found to be comparable with the results of silymarin. However formulation F - III has demonstrated significantly better result as compared to formulations F - I and F - II when biochemical parameters are taken into consideration. This can only be confirmed in further studies for chronic hepatotoxicity and mechanism of hepatoprotection.

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