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Formulation Development and Hepatoprotective activity of Hydro-alcoholic extract of *Abutilon indicum* and *Phyllanthus niruri* against paracetamol induced liver toxicity in albino rats

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Abstract

The term "hepatic disease" refers to a broad category of conditions that affect the human liver's tissues, structures, and cells. The liver performs a great deal of vital processes, therefore there are many opportunities for anything to go wrong. Inflammation is one of the most frequent causes of liver illness and is frequently brought on by alcohol abuse, a bad diet, or even malnourishment. The most significant health concern that affects the drug control board, the pharmaceutical industry, and medical professionals is medication-induced liver damage or dysfunction. The United States Acute Liver Failure Study Group states that over half of cases of acute liver failure are due to drug-induced liver injury, which includes paracetamol overdose-induced hepatotoxicity. The present paper deals with the formulation of polyherbal tablet containing hydro-alcoholic extract of *Abutilon indicum* Leaves and *Phyllanthus niruri* Fruits. The formulated tablet was evaluated and screened for Hepatoprotective activity against paracetamol induced liver toxicity in albino rats. The results indicate that prepared polyherbal tablet showed significant hepatoprotective activity as compared to the hepatotoxic control.

Keywords: Liver disorders, Medicinal Plants, Paracetamol induced

Introduction

The field of herbal medicine has grown exponentially in the past several years, and due to their natural origins and low side effects, these medications are becoming more and more popular in both developed and developing nations. Medicinal plants, minerals, and organic materials are the source of many commonly used traditional medications. Many medicinal plants known as rasayana, which have been utilised for over a millennium, are included in herbal remedies used in Indian traditional medical systems. The majority of medical professionals in Indian systems create and administer their own concoctions. 21,000 plants are registered by the World Health Organisation (WHO) as being used medicinally worldwide. Of these 2500 species, 150 are employed commercially on a somewhat regular basis in India.. [1-3]

Indian Indigenous medicinal plants are most widely used for the treatment of several diseases either in alone or in combination in raw as well as their extract. Synthetic hepatoprotective agents can produce several serious effects and also they are not suitable to use during pregnancy. In this light herbals are preferred in the treatment of liver disorders. Ancient ayurvedic literature reveals that the selected plants i.e., *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits) have been widely used in the treatment of liver disorders. These plants have been extensively used in ayurveda and traditional system of medicine for the treatment of liver disorders and found to be efficient and inexpensive as compared to synthetic drugs and not evaluated scientifically in combination as polyherbal tablet. Therefore, it was worthwhile to investigate the hepatoprotective activity of polyherbal tablet containing hydr0oalcoholic extract of *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits).

Material and Methods

Collection of herbs and their authentication

Abutilon indicum (Leaves) and *Phyllanthus niruri* (Fruits) were collected from local sites of Malwa region of Madhya Pradesh, India during October 2023 and identified morphologically, microscopically and compared with standard pharmacopoeial monograph and authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, Visiting Professor, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot./AI-L/12; J/Bot/PN-F/13 were allotted to the selected plant parts.

Extraction of selected herbs

The shade dried coarsely powdered plant material (250 gms) of plant viz., *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits) were loaded in Soxhlet apparatus and was extracted with ethanol: water for 48 hour. After completion of extraction, the solvent was removed by evaporation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined. [8-9]

Pharmacological screening

Acute Toxicity Studies of Extracts & Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization. IEAC approval.

Development of Polyherbal Formulation

Preparation of Polyherbal formulation (Tablet)

Polyherbal formulations table (PFT) containing hydro-alcoholic extract of plant *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits) was prepared by wet granulation method using

suitable excipients like microcrystalline cellulose, starch, crospovidone, aerosil and magnesium stearate.[7-8] The composition of PFT was given in table 1.

Table 1: Composition of Polyherbal formulation (Tablet)

Ingredients	**Quantity**
HAEAIL	100
HAEPNF	100
Microcrystalline Cellulose	125
Starch	50
Crospovidone	20
Granulation	
Water	q.s.
Prelubrication	
Starch	25
Aerosil	10
Talc	15
Lubrication	
Magnesium Sterate	5
Total weight (mg)	500

Evaluation of polyherbal formulation (Tablet) [7-9]

Appearance

The prepared herbal tablets were evaluated for their color and appearance. In this study color, odor, taste were noted down.

Hardness

Randomly five tablets were taken out from each batch and crushing strength was determined using Monsanto tablet hardness tester.

Friability

Randomly 25 tablets were taken and weighed out and was placed in Electrolab friabilator and was rotated at 25 rpm for 4 mts to determine the frability. The percentage friability was calculated by using formula

$$\%F = (1-WI/WF)*100$$

Where, WI=Initial weight of the 25 tablets; WF=Final weight of 25 tablets

Weight Variation

Randomly selected 20 tablets were evaluated for weight variation as per IP 2018.

Disintegration Time

Randomly 6 tablets were taken from each batch and were placed in USP disintegration apparatus using 0.1 N HCl at 37^0C. The time was noted down when the tablet get disintegrates completely.

Hepatoprotective activity of Polyherbal Formulatio (Tablet)

Experimental Animal

Albino rats (200-250 g) used in the present studies was procured. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.

Paracetamol Induced Model

The rats were divided into 7 groups of 6 animals in each. [18-19]

S/No.	Group	Treatments
1.	Group I (Normal)	Received vehicle gum acacia (5mg/kg p.o) for 7 days
2.	Group II (Control)	Received vehicle gum acacia (5 mg/kg p.o) for 7 days once daily and paracetamol 500mg/kg once daily
3.	Group III (Standard)	Received silymarin as standard (50 mg/kg) for 7 days once daily and paracetamol 500mg/kg once daily
4.	Group IV	Received PFT (100 mg/kg) once daily and paracetamol 500mg/kg once daily
5.	Group V	Received PFT (200 mg/kg) once daily and paracetamol 500mg/kg once daily

On the seventh day, the blood samples were collected via orbital sinus puncture for the estimation of biochemical marker enzymes and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters. Then the liver was carefully isolated and cleaned off extraneous tissue and preserved in 10% neutral formalin and then subjected to histopathological studies. [13-14]

Statistical Analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnette multiple Comparisons test. Statistically significance of * $P<0.01$, ** $P<0.001$, when compared with respective control. All values are expressed as mean ± SEM.

Results and Discussion

The hydroalcoholic extracts of *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits) were screened for acute toxicity study by OECD guideline no. 423 for determination of LD_{50}. The LD_{50} was 2000 mg/kg, therefore, ED_{50} was 200 mg/kg. Polyherbal formulation (tablet) containing hydroalcoholic extracts of *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits), were evaluated for appearance, hardness, friability, weight variation and disintegration time. The physical appearances of PFT were given in table 2. The results obtained suggest that formulation do not have any tablet defects. The evaluation parameters of PFT were shown in table 3. The results obtained indicates that the data obtained are within the limit as per IP.

Rats treated with silymarin, PFT (at the dose of 100 & 200 mg/kg bw respectively) formulations showed noticeable improvement in histopathological parameters. The results were presented in table 4. The results were found to be more promising at the dose of 100 mg/kg bw than 200 mg/kg bw in pacarematol induced liver toxicity when compared to standard drug silymarin. Hence the formulations have prominent action on paracetamol-induced liver damage. Moreover, at necropsy, livers of rats treated with paracetamol appeared degeneration in hepatocytes, hepatic cell injury, focal necrosis, congestion in central vein, vascular swelling, and Kupffer cell proliferation. Furthermore, no gross pathological findings were noted in the livers of the other groups of rats.

Table 2: Physical Parameters (Appearance) of Polyherbal Formulation Tablet

Formulation Code	Physical Parameters (Appearance)			
	Color	**Odor**	**Taste**	**Shape**
PFT	Light green	Characteristic	Characteristic	Circular biconvex

Table 3: Evaluation Parameters of Polyherbal Formulation Tablet

Formulation Code	Parameters			
	Weight variations (%)	**Hardness (kg/cm^2)**	**Friability (%)**	**Disintegration time (mts)**
PFT-1	±3.52	4.74±0.08	0.31±0.24	8.66±0.02

Note: All values are expressed in Mean±SD, n=3

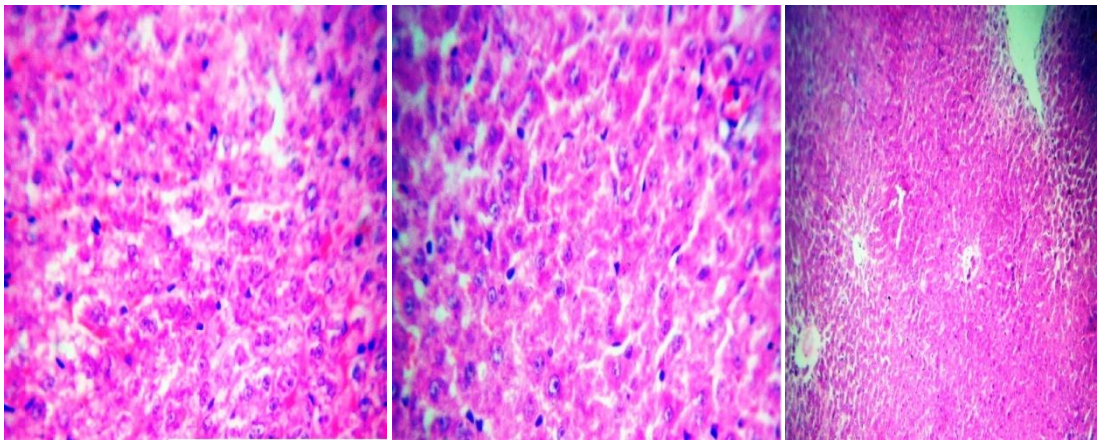
Table 4: Effect of Polyherbal formulation table on Biochemical Parameters in Paracetamol Induced Hepatic Injury in Rats

Treatment	Total Bilirubin (mg %)	Direct Bilirubin (mg %)	SGOT (µ/min/l)	SGPT (µ/min/l)	ALP (µ/min/l)
Normal	0.44 ± 0.21	0.43 ± 0.64	183.02 ± 2.1	77.40 ± 2.43	192.0 ± 6.2
Induced (PCM 2g/kg)	8.61 ± 2.46	7.45 ± 8.60	345.41± 10.42	153.7± 8.44	358.22±8.85
Standard (Silymarin 50mg/kg)	0.53 ±4.39**	0.49 ±0.19**	197.07±9.43**	88.07±8.79**	199.21 ±10.61**
PFT-1 (100 mg/kg)	0.58 ±0.01**	0.55 ±0.22**	203.14± 6.10**	94.02±6.01**	204.02± 8.32**
PFT-1 (200 mg/kg)	0.61 ±0.11**	0.59 ±0.27**	207.27± 8.39**	100.12±8.11**	206.13± 8.01**

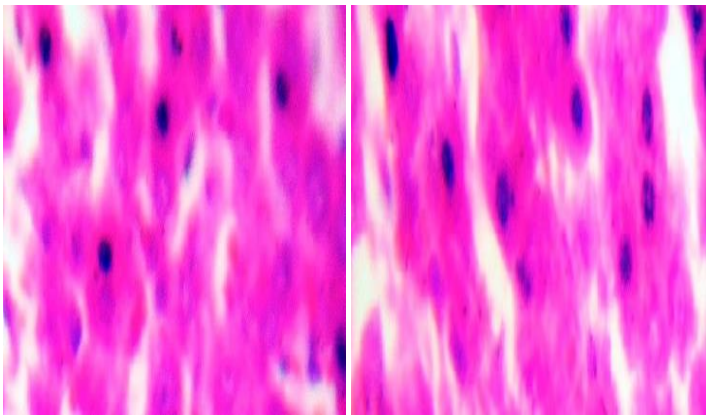
Note: Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.

Conclusion

The liver disorders are very common and alarming. In the present work formulation development, evaluation and hepatoprotective activity of *Abutilon indicum* (Leaves) and *Phyllanthus niruri* (Fruits) against paracetamol induced liver toxicity in albino rats were investigated and the results the formulated polyherbal tablet at the test dose of 100 mg.kg bw showed significant hepatoprotective activity



Normal Paracetamol induced (500mg/kg) Silymarin (50mg/kg)



PFT 100 mg/kg PFT 200 mg/kg

Fig. 1: Histopathologic Section of Liver of Rats in Paracetamol induced Hepatotoxicity of Normal, Control, Standard, PHT

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