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POTENTIAL HEPATOPROTECTIVE EFFECT OF HERBAL FORMULATION ON PARACETAMOL INDUCED HEPATOTOXICITY IN MICE

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ABSTRACT

The medicinal plants play very important role in different health care systems. Traditional system of medicine recommended various hepatoprotective agents and preparations to treat hepatic disorders. Polyherbal formulation F1 was developed and evaluated for its hepatoprotective effects using acute liver toxicity with paracetamol intoxication in mice. The formulation F1 is composed with *Andrographis paniculata* (Kalmegh), *Boerhavia diffusa* (Punarnava), *Eclipta alba* (Bhringraj) and *Picrorhiza kurroa* (Kutki). The hepatoprotective assessment was done with serum biochemical parameters as serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin, triglyceride and lipid peroxidation. F1 showed significant hepatoprotective activity at dose of 400 mg/kg, which was comparable to standard drug: silymarin at 50 mg/kg. F1 markedly reversed the toxic effects of paracetamol as reflected by the modulation in these biochemical estimations. The result of the study strongly indicates that formulation F1 has a potent hepatoprotective action against paracetamol induced hepatic damage in mice.

Keywords: Polyherbal formulation, Hepatoprotective effect, Paracetamol, Liver function test

INTRODUCTION

Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of its functions include metabolism and disposition of chemicals (xenobiotics) to which the organ exposed directly or indirectly metabolism of lipids, carbohydrates and proteins, blood coagulation and immunomodulation. Conventional or synthetic (allopathic) drugs used in the treatment of liver

disease are sometimes inadequate and can have serious adverse effects. Therefore, there is a worldwide trend now to go back to traditional medicinal plants [1]. Many natural products of herbal origin are in use for the treatment of liver ailments like acute or chronic inflammation, toxin/drugs- induced hepatitis, cirrhosis and hepatitis after viral infection [2]. Nevertheless, a large number of herbal drugs or their formulations have not been studied for their protective effects on different organs.

Acetaminophen (N-acetyl-p-aminophenol) or Paracetamol is a commonly used analgesic and proved to be very safe therapeutics. However, in overdoses it causes a fulminating hepatic necrosis [3]. The overdoses of paracetamol in experimental animals to produce hepatotoxicity [4]. For instance, *Andrographis paniculata*, *Boerhavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* have not been reported to have hepatoprotective effects. These drugs are very potent for the treatment of various ailments in the traditional system of medicine. The herbal formulations of these medicinal plants were used to treat the hepatic centrilobular necrosis produced by paracetamol in albino mice.

Materials and Methods

Collection and Processing of plant materials

The fresh leaves of *Andrographis paniculata*, root of *Boerhavia diffusa*, whole plant of *Eclipta alba* and rhizome of *Picrorhiza kurroa* were collected from the botanical garden of Indian Institute of Integrative Medicine (Regional Research Laboratory), Jammu. It was botanically identified and authenticated. A voucher specimen (Collection No. 50028, 50029, 50030 and 50031) has been kept in our laboratory for future reference. The plants were shade dried, powdered, sieved through 40 mesh and stored in a tightly closed container for further use.

Preparation of the Plant Extract

The four powdered plant materials (200gm) was extracted successively with different solvents like Methanol (*Andrographis paniculata*), 80% ethanol (*Boerhavia diffusa*), 80% ethanol (*Eclipta alba*) and 50% ethanol (*Picrorhiza kurroa*) for twenty hours in round bottom flask and filtered. The marc was macerated with same solvent for 24 hour and filtered. This process was repeated and all the filtrates were collected and concentrated in rotary evaporator. The remaining solvent was evaporated in a water bath to obtain the extract.

Animal Used

Swiss albino mice weighing 20-25gm were procured from Indian Institute of Integrative Medicine (Regional Research Laboratory) Jammu Tawi, India. They were maintained under uniform laboratory conditions in standard steel cages and provided with food (Lipton India Ltd., Bombay, India) and water ad libitum. The experiments were conducted according to the norms approved by Institutional Animal Ethics Committee guide lines for animal care and were adhered to as recommended by the Indian National Science Academy, New Delhi (1992).

Preparation of Formulation

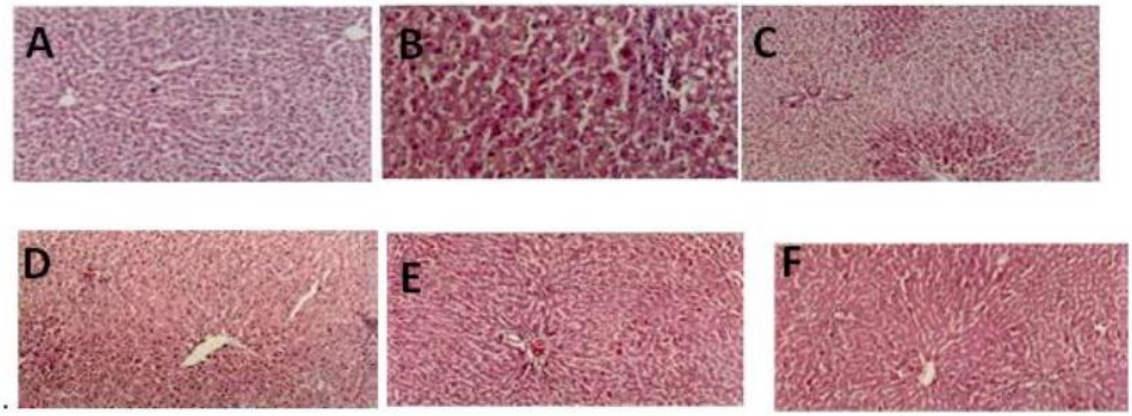
The extracts of the plants parts of *Andrographis paniculata*, *Boerhavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* were employed to prepare formulations in different proportions as 1:1:1:1, 1:2:1:2, 2:1:2:1 referred as Formulation 1 (F1), Formulation 2 (F2) and Formulation 3 (F3) respectively.

Experimental Protocol

Paracetamol (Sigma-Aldrich, USA) was suspended in distilled water and was administered orally at a dose of 500mg/kg bodyweight [5]. F1 was administered to mice

continuously for three days. On day fourth the mice was administered with F1 followed by administration of toxicant after two hour. Again formulation 1 was administered on the fifth day, the mice were sacrificed to collect the blood and liver samples for blood analysis [6]. The experimental animals were divided into six groups, each group comprising six animals.

Group 1: Control mice fed with standard diet.
Group 2: Paracetamol (500mg/kg, p.o.)
Group 3: Formulation 1 (100mg/kg, p.o.)
Group 4: Formulation 1 (200mg/kg, p.o.)
Group 5: Formulation 1 (400mg/kg, p.o.)
Group 6: Silymarin (50mg/kg, p.o.)



Hepatic histological finding against hepatic lesions induced by Paracetamol- A: Normal Group, B: Paracetamol alone, C: Paracetamol (500mg/kg) + F1 formulation (100mg/kg), D: Paracetamol (500mg/kg) + F1 formulation (200mg/kg), E: Paracetamol (500mg/kg) + F1 formulation (400mg/kg), F: Paracetamol (500mg/kg) + Silymarin (50mg/kg, p.o.).

Biochemical Estimations

At the end of the experimental period, the animals were killed by cervical dislocation. Blood was collected in the glass tubes from orbital sinus to obtain haemolysis free clear serum for the analysis for the estimation of (SGOT) Serum Glutamate Oxaloacetate Transminases and (SGPT) Serum Glutamate Pyruvate Transminases [7]. Triglycerides [8]. All the animals were sacrificed by decapitation and livers were quickly excised freed from any adhering tissues, washed and perfused with chilled normal saline, minced and homogenized in ice bath using Potter-S-homogenizer (B. Braun, MelsungenAG, Germany, 1100 rpm for 2 min) in chilled 10mM Tris–HCl buffer (pH 7.4) to obtain 10% liver homogenate for the estimation of (GSH) glutathione [9]. (LP) lipid peroxidation [10] and estimation of Albumin was used standard kit (Bayer Diagnostic Ltd., Gujarat, India).

Histopathological Investigation

The liver tissues were excised out, washed with the cold saline, fixed in 10% buffered formalin for 12 hours and processed and stained with hematoxylin and eosin dye for photomicroscopic observations.

Statistical analysis

The data obtained were analyzed by One Way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using computerized program. p value <0.05 was taken as the criterion of significance.

Results

The findings of ALT, AST, ALB, TG, LP and Glutathione have been summarized in the (Table 1). It is seen that administration of paracetamol at a dose of 500mg/kg; p.o. for 5 day caused significant rise (p≤0.01) in level of serum marker enzymes such as AST, ALT, Triglycerides and Lipid peroxidation levels. Silymarin (p≤0.01) significantly reduced these levels near to normal. A significant (p≤0.01) decreased was observed in the serum levels of AST, ALT, Triglycerides and Lipid peroxidation, along with Significantly Increased levels of Albumin and Glutathione levels, in the animals treated with different doses (100, 200 and 400 mg/kg; p.o) of F1 formulation and shows dose dependant activity. At the dose of 400 mg/kg; p.o. F1 formulation showed comparable activity with the standard drug Silymarin (p≤0.01).

Histopathological studies revealed that paracetamol caused focal necrosis, fatty changes, ballooning degeneration and infiltration of lymphocytes around the central vein, as compared to normal, paracetamol treated rats which shows normal central vein, portal triad and hepatocytes as shown in (Fig. A & B). Necrosis induced by paracetamol was markedly prevented by treatment with dosed of F1 formulation at the dose of (100, 20 and 400 mg/kg; p.o) (Fig. C, D & E) and 50 mg/kg; p.o dose (Fig. F) of Silymarin showed occasional area of cell degenerations with normal central vein and portal triad around the central vein with no fatty changes. Histogram shows F1 formulation at the dose of 400 mg/kg; p.o. shows comparable result with the standard drug Silymarin.

Table 1:

Serum						**Liver homogenate**	
Treatments	Dose Mg/kg	ALT µmole/min/lt	AST µmole/min/lt	ALB gm%	TG mg%	GSH n mole MDA/g/liv	LP µmole MDA/g liv
Vehicle	----	101.41±4.36	108.18±6.10	3.06±0.03	96.21±4.68	5.18±0.29	103.35± 6.20
Vehicle + paracetamol	500	1923.47±159.9	909.91±45.46^{C}	2.47±0.13^{A}	129.66±9.63^{A}	2.87±0.11^{C}	147.26±8.70^{B}
F1 + paracetamol	100	1350.58±99.07^{a}	648.19±54.55^{b}	2.65±0.08	119.58±4.52	3.74±0.24^{b}	131.54±6.62
F1 + paracetamol	200	1227.36±88.62^{b}	634.49±35.95^{c}	2.70±0.90	117.97±5.76	3.81±0.32^{a}	128.09±4.19
F1 + paracetamol	400	1162.94±69.01^{b}	545.19±36.05^{c}	2.72±0.07	115.19±10.36	3.96±0.23^{b}	125.10±3.04^{a}
Silymarin + paracetamol	50	1062.33±109.6	520.34±37.69	2.78±0.07	111.68±9.67	4.24±0.34	121.61±4.35

(Values as Mean ± SE, N= 6)

P Value paracetamol Vs Control- A ≤ 0.05, B ≤ 0.01, C ≤ 0.001, P value treatment Vs paracetamol- a ≥ 0.05, b ≥ 0.01, c ≥ 0.001

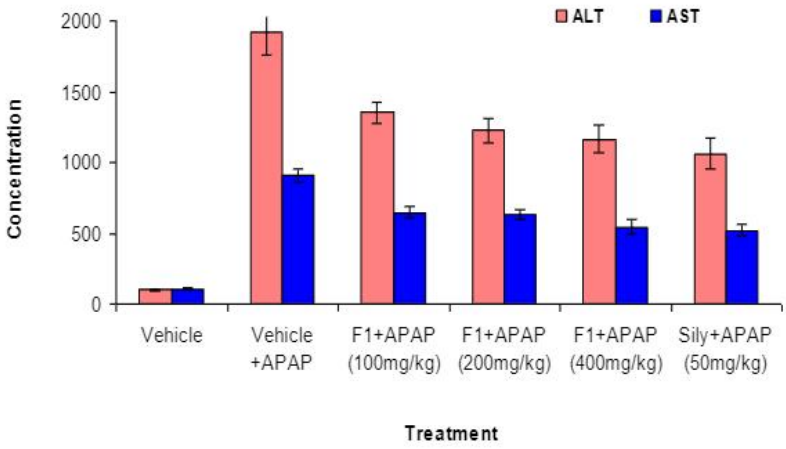


Figure1. Effect of Formulation F1 on transaminases levels in paracetamol induced hepatotoxicity.

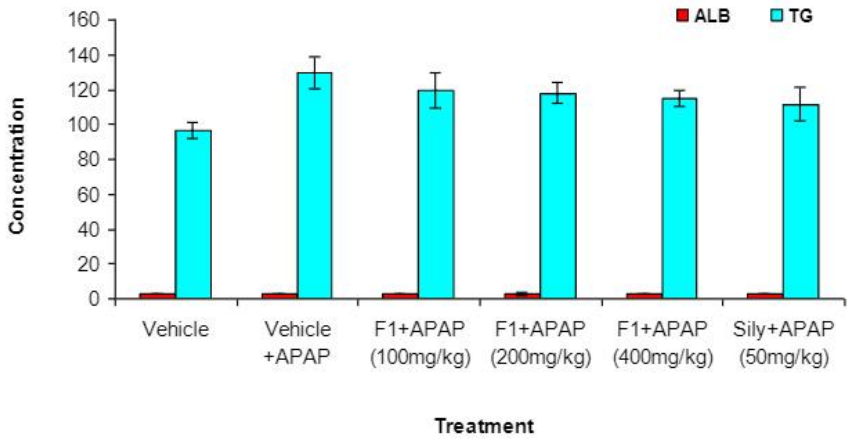


Figure 2. Effect of Formulation F1 on albumin and triglyceride levels in paracetamol induced hepatotoxicity.

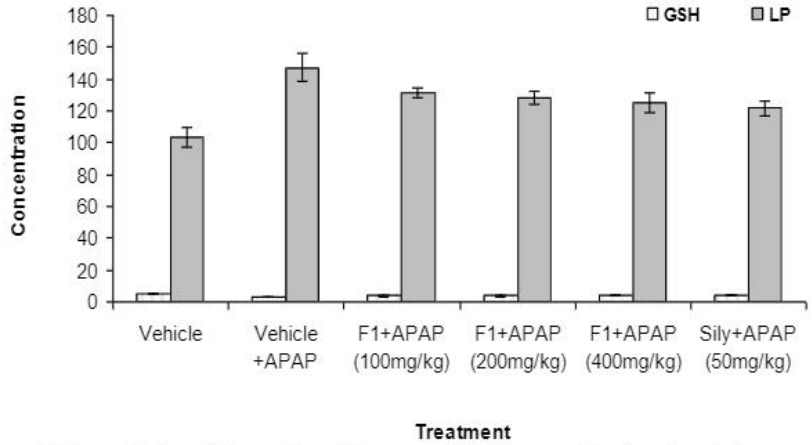


Figure 3. Effect of Formulation F1 on glutathione and lipid peroxidation levels in paracetamol induced hepatotoxicity.

Table 2: Percentage protection of Formulation F1 against paracetamol induced hepatotoxicity

Serum						Liver homogenate	
Treatments	Dose mg/kg	ALT µmole/min/lt	AST µmole/min/lt	ALB µmole PNP/min/lt	TG mg%	LP µmole MDA/g liv	GSH N mole/g/liv
Formulation 1	100	31.44%	38.10%	30.50%	30.13%	35.80%	37.66%
Formulation 1	200	35.20%	34.35%	38.98%	34.94%	43.65%	40.69%
Formulation 1	400	41.47%	49.44%	42.37%	43.25%	50.46%	47.18%
Silymarin	50	47.26%	48.59%	52.54%	53.75%	59.30%	58.41%

Discussion

Paracetamol (N-acetyl p-amino phenol) is a widely used analgesic and antipyretic drug, known to cause hepatotoxicity in experimental animals and humans at high doses. It is mainly metabolized in liver to excreatable glucuronide and sulfate conjugates. However, hepatotoxicity of paracetamol has been attributed to formation to toxic metabolites, when a part of paracetamol is activated by hepatic Cytochrome P450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as conjugates. Overdoses of paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis. Damage to the structural integrity of liver is reflected by an increase in level of Serum Transminases; these are cytoplasmic in location and are released into circulation after cellular damage [11]. Present study raveled a significant rise in the level of serum markers enzymes viz. AST, ALT, Triglycerides and Lipid peroxidation level on exposure to paracetamol, indicating considerable hepatocellular injury. Administration of F1 formulation at dose of 100, 200 and 400 mg/kg; p.o. dose level attenuated the increased level of the serum enzymes, along with Significantly Increased levels of Albumin and Glutathione levels, produced by paracetamol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

Hepatic GSH depletion or even extra hepatic GSH depletion can provide useful information on the protective role GSH against toxic foreign compounds. Thus GSH, be regarded as an endogenous protective agent against drugs[12].Paracetamol treated animals showed decrease in level of GSH than normal. While treatment of F1 formulation enhanced the GSH level significantly. In a dose response study, it was found that F1 formulation at a dose of 400 mg/kg; p.o. could almost protect the liver from toxic injury, which was better, when compared with the standard drug, Silymarin. In Conclusion, the results of the present study shows that F1 formulation could prevent oxidative liver damage by restoring enzyme levels to near normal.

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