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Hepatoprotective effect of polyherbal preparation against paracetamol-induced liver toxicity in rats

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Abstract

Hepatoprotective activity of Polyherbal preparation against Paracetamol-induced hepatic damage in rats was observed. The healthy control (normal), control, and standard drug Silymarin-treated groups were also maintained for the comparison. The liver marker enzymes SGOT, SGPT, ALP and total Bilirubin were assessed in all the experimental groups. The changes in liver function parameters were significant in comparison to control group and the observed efficacy was comparable to standard drug. The efficacy of the polyherbal preparation was found to be dose dependent. The histopathology study of liver also supports the presence of hepatoprotective activity in polyherbal preparation by showing improved cytoarchitecture of liver cells in the treated groups.

1. INTRODUCTION

Hepatotoxicity resulting from liver damage which plays a pivotal role in amendable various physiological processes in the body, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles[1]. Paracetamol promote development of free radicals and subsequent lipid peroxidation damages the membranes of liver cells and organelles and causing the swelling and necrosis of hepatocytes. Paracetamol is widely used an antipyretic and analgesic, but higher doses produced acute liver damage[2]. Various newly advanced drugs have been used for treatment of liver diseases; however, these drugs produced destructive side effects. For that purpose, further research on herbs that could potentially substitute the chemical-based drugs is very crucial as several medicinal herbs have been shows hepatoprotective properties and offers a comprehensive coverage for the treatment of virtually every manifestation of liver dysfunction[3]. Polyherbal preparation which contain *Andrographic paniculata* (kalmegh), *Night Jaismine* (parijat) and *Annona squamosal* (sitaphal) that is currently being investigated for its potential pharmacological activities. *Andrographic paniculata*, *Night Jaismine* and *Annona squamosal* are used in traditional system of medicine for various clinical conditions like antioxidant, liver disease, anti-inflammatory, wounds and ulcer[4, 5]. From our literature review, no attempt has been made to study the hepatoprotective Polyherbal potential of *Andrographic paniculata*, *Night Jaismine* and *Annona squamosal* had shown that the antioxidant activity played significant role in the mechanisms of hepatoprotective activity.

The aim of study has been designed to evaluate the hepatoprotective activity of polyherbal preparation in the experimental animal models of Paracetamol-induced liver toxicity in rats.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Paracetamol (Sigma chemicals, USA) and Silymarin (Micro labs, India) was used in present study. All other chemicals and other biochemical used in the experiments were of analytical grade from different firms.

2.2 Collection of plant material

The plants were collected from wild plants or cultivated plants. The plants were collected in the month of September to November from local area of Bhopal, India.

2.3 Preparation of plant extracts

The plant materials were shade dried for 7 days and coarsely powdered. The powder was mixed thoroughly with 6 times the volume of DMSO in water and stirred continuously until the volume reduced to 1/3rd. The extract was filtered with muslin cloth. The residue was re extracted. The filtrate was mixed and evaporated in a water bath till it reached a paste consistency consistency. The extract was stored in refrigerator till further use. The alcoholic extract of the plants *Andrographic paniculata*: *Annono squamosa* and; *Night jaismine* were mixed in the ratio1; 1:1 and Polyherbal preparation was used for the study.

2.4 Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.5 Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD)[6]. Animals were kept fasting providing only water, *Andrographic paniculata*, *Night Jaismine*, *Annona squamosal* and its preparation (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible hepatoprotective effect.

2.6 Experimental

2.6.1 Paracetamol induced hepatotoxicity

Animals were randomly divided into different groups comprising six rats each. Animal of Group I served as normal received distilled water, Group II was control, received vehicle. Animal of Group III received standard silymarin 50 mg/kg p.o. Groups IV -V were treated with *Andrographic paniculata* (100 and 200 mg/kg, p.o.), Groups VI-VII were treated with *Night Jaismine* (100 and 200 mg/kg, p.o.), Groups VIII-IX were treated with *Annona squamosal* (100 and 200 mg/kg, p.o.) and Groups X -XI were treated with Polyherbal preparation (50 and 100 mg/kg, p.o.) for 5 days[7]. On the third day, paracetamol suspension (5% gum acacia) was administered in a dose of 3 g/kg Body weight, orally to all groups except normal.

Group –I: Normal control (distilled water.)
Group –II: Paracetamol [PCM, 3g/kg p.o.]
Group -III: Silymarin (50mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group -IV: *Andrographic paniculata* (100mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group -V: *Andrographic paniculata* (200mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –VI: *Night Jaismine* (100mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –VII: *Night Jaismine* (200mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –VIII: *Annona squamosal* (200mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –IX: *Annona squamosal* (200mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –X: Polyherbal preparation (50mg/kg, p.o.) + PCM (3g/kg, p.o.)
Group –XI: Polyherbal preparation (100mg/kg, p.o.) + PCM (3g/kg, p.o.)

At the end of the experimental period the animals were sacrificed. The blood and liver tissue were used for the studies. Blood was collected after 48 h. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes and utilized for the estimation of various biochemical parameters including Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total bilirubin[8-9.]

2.6.2 Histopathological investigation

Small pieces of liver tissues of each group of animals were stored in solution of commercial formaldehyde for histopathological studies.

2.6.3 Statistical analysis

Each experimental value is expressed as the Mean ± SEM. Statistical calculations of the data were performed using ANOVA analysis. A probability of $P < 0.05$ was considered as significant.

3. RESULTS

Table 1 shows the results of SGOT, SGPT, ALP and total bilirubin in all groups of rat. The present study revealed that, PCM administration showed significant elevation in SGOT, SGPT, ALP and total bilirubin which was significantly (P<0.5) reduced by treatment with Polyherbal preparation, other plants extract and Silymarin 50 mg/kg p.o. treatment onwards till the end of the study (Fig- 1- 4).

Table-1: Effect of Polyherbal preparation against PCM Induced Hepatotoxicity

Group	SGOT	SGPT	ALP	Total Bilirubin
Group –I	92.08 ± 8.890	41.29 ± 5.607	116.38 ± 8.281	0.35 ± 0.063
Group –II	220.37 ± 15.073	129.35 ± 13.131	257.27 ± 16.205	1.06 ± 0.068
Group –III	106.80 ± 9.306***	53.10 ± 6.788***	131.02 ± 10.680***	0.47 ± 0.061***
Group –IV	151.42 ± 9.764**	79.23 ± 6.231**	155.67 ± 12.560**	0.75 ± 0.055*
Group –V	139.55 ± 10.431**	66.10 ± 5.896***	150.46 ± 14.450**	0.69 ± 0.060**
Group VI	149.98 ± 11.356**	80.00 ± 4.966**	156.56 ± 11.570**	0.70 ± 0.059*
Group –VII	133.77 ± 10.894***	69.45 ± 5.468***	149.23 ± 13.560***	0.68 ± 0.060**
Group –VIII	148.65 ± 10.457**	78.90 ± 7.014**	147.87 ± 12.298***	0.70 ± 0.062*
Group –IX	135.22 ± 9.255***	64.67 ± 6.451***	140.65 ± 14.486***	0.65 ± 0.057**
Group X	127.11 ± 10.130***	60.99 ± 6.762***	138.77 ± 13.988***	0.52 ± 0.064***
Group –XI	118.55 ± 15.191***	57.90 ± 5.848***	134.22 ± 14.298***	0.50 ± 0.058***

*Values are expressed as MEAN±SD at n=4, One way ANOVA followed by Dunnett's test, *P<0.05 compared to the PCM control*

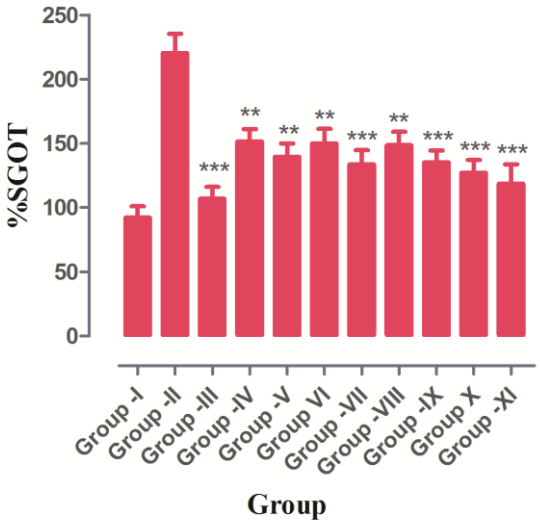

Fig. 1: Effect of plant and formulation on %SGOT level in paracetamol induced hepatotoxicity in rats

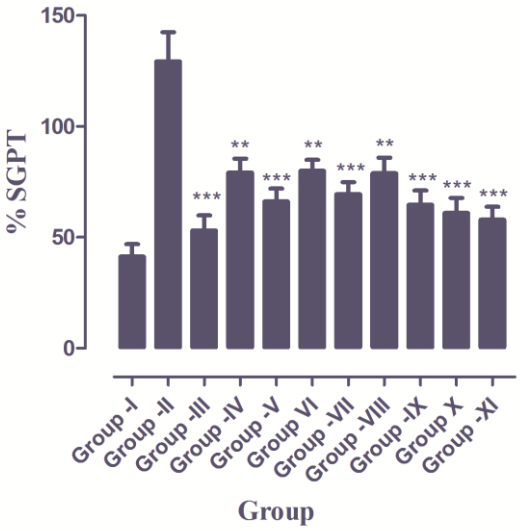

Fig. 2: Effect of plant and formulation on %SGPT level in paracetamol induced hepatotoxicity in rats

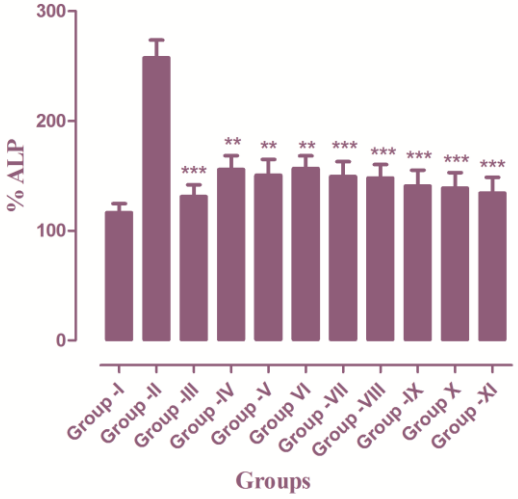

Fig. 3: Effect of plant and formulation on %ALP level in paracetamol induced hepatotoxicity in rats

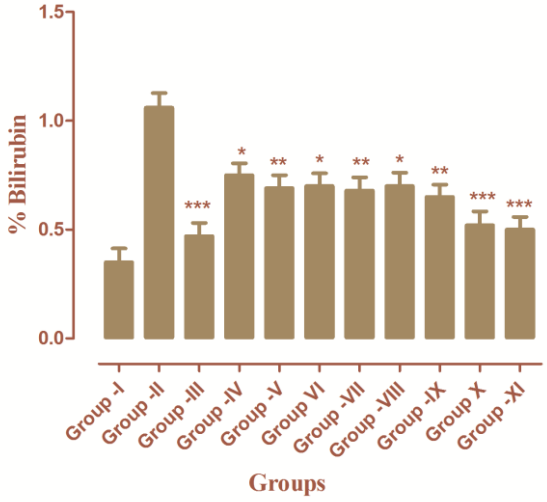

Fig. 4: Effect of plant and formulation on % Bilirubin level in paracetamol induced hepatotoxicity in rats

3.1 Histopathological studies

Histopathological changes of liver are given in Fig.5. Histology of the liver sections of normal control animals showed normal liver architecture with well brought out central vein well-preserved cytoplasm and prominent nucleus and nucleolus (Fig. 5A). The liver samples of control animals showed feathery degeneration, micro and macro cellular fatty changes, and inflammatory cells around portal tract (Fig. 5B). The control + standard treated animals also showed a good protection from inducer changes in the liver (Fig. 5C). The histopathological examination clearly revealed that the hepatic cells, central vein, and portal triad were almost normal in Polyherbal preparation and other groups (Fig.5D-K).

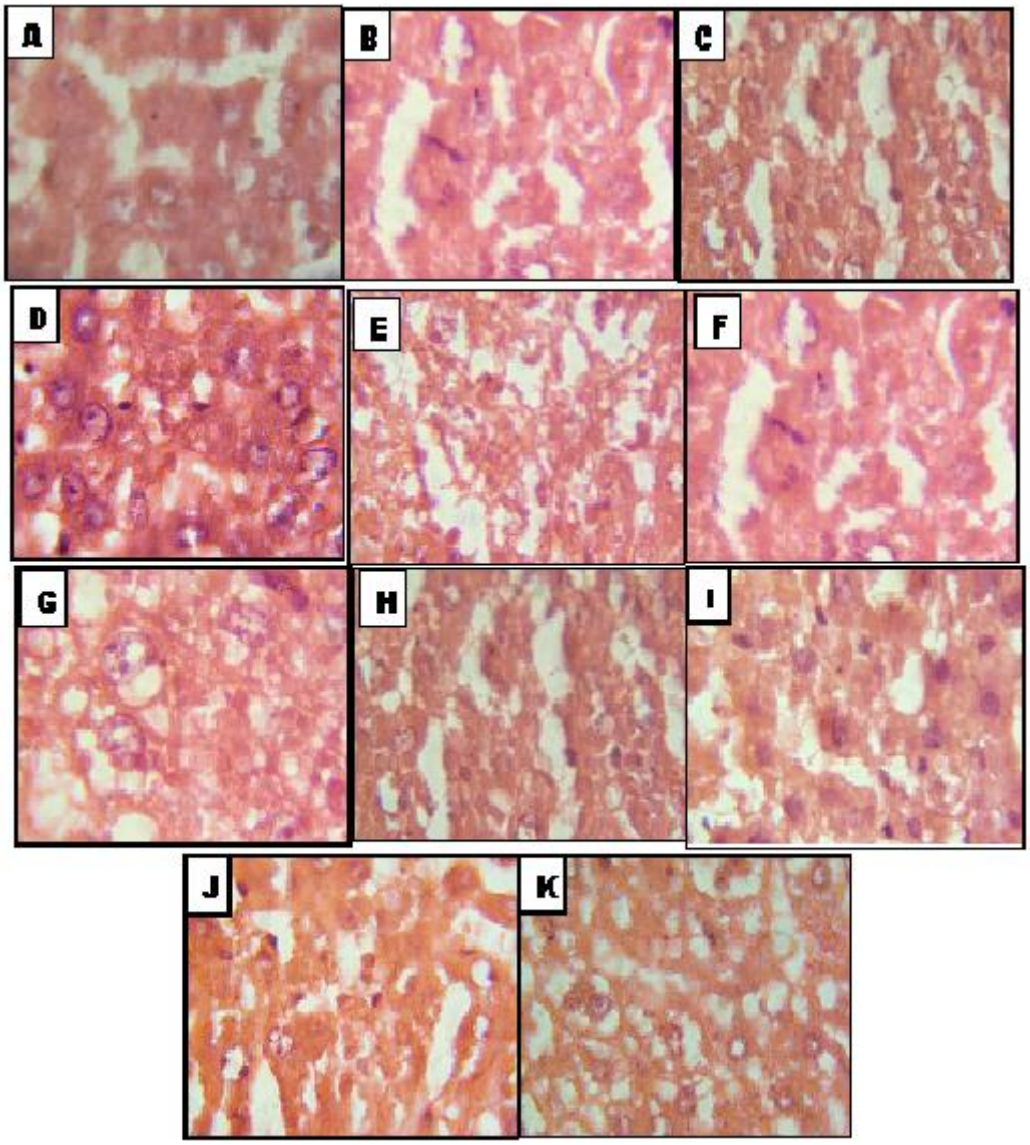

Fig.5: Histological changes

4. DISCUSSION

The liver is a versatile organ in the body with regulation of chemical environment. Consequently, liver damage caused by a hepatotoxic agent is of severe significance[10]. Paracetamol is an antipyretic and analgesic agent, which is safe in therapeutic doses, but can produce fatal hepatic necrosis in toxic doses[11-12]. It is processed in the liver to extractable glucuronide and sulphide conjugates[13]. Hepatic injury is the leaking of cellular enzymes into the plasma due to disruption caused in the transport purposes of hepatocytes[14-15].When liver cell cytoplasm is injured, a variety of enzymes situated normally in cytosol are released into the blood, thus causing increased enzyme levels in the serum. Liver enzymes such as SGOT, SGPT, ALP and total bilirubin are considered to be biochemical markers for assessing liver function. Hepatotoxicity is evidenced by an elevation of the serum marker enzymes[16]. The Polyherbal preparation significantly ($P<0.05$) reduced the liver enzymes levels in experimental animals shows that combined therapy has hepatoprotective action (Table 1). During the experimentation, Wistar rats did not

show any mortality or any other adverse effects when the rats fed orally with Polyherbal extracts at the doses of 50- 100 mg/kg/day. Thus the Polyherbal preparation has a good margin of protection. In conclusion, Polyherbal preparation show pharmacological potential against PCM induced hepatotoxicity due to synergism of plant extracts.

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REFERENCES

1.Thamizh Selvam N, Venkatakrishnan V et al. "Hepatoprotective activity of methanolic extract of *Syzygium jambos* (Linn.) leaf against paracetamol intoxicated Wistar albino rats". *AYU,* 2013, 34: 305-307.

2.Akindele AJ, Ezenwanebe KO et al. "Hepatoprotective and in vivo antioxidant effects of *Byrsocarpus coccineus Schum*. and Thonn. (Connaraceae)". *J. Ethnopharmacol*, 2010, 129: 46–52.

3.Kallappa SG and Jambilingappa LK. "Evaluation of hepatoprotective activity of aqueous extract of *Azadirachta indica* (neem) leaves against paracetamol induced hepatotoxicity in albino rats". *Pharmacologyonline,* 2011, 2: 96–108.

4.Sharma PC, Yelne MB et al. Database on Medicinal plants used in Ayurveda. CCRAS, 2002, 4: 34-8.

5.World Health Organization. WHO Monographs on selected medicinal plants. Geneva: AITPBS Publications and Distributors, 2002, 2: 12-24.

6.OECD. Guideline for Testing of Chemicals-Acute Oral Toxicity-Acute Toxic Class Method. Paris: OECD, 2001.

7.Chattopadhayay RR, Bandyopadhyay M. Possible Mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract against paracetamol induced hepatic damage in rats: Part III. *Indian J Pharmacol*, 2005, 37:184-5.

8.King J. The Hydrolases Acid and Alkaline Phosphatase. In Practical Clinical Enzymology. Edited by Van D. London: Nortstand Company Ltd, 1965: 191-208.

9. Malloy HT, Evelyn KA. The determination of bilirubin. *J.Biol Che,* 1937, 119: 481.

10.Senthilkumar R, Chandran R et al. "Hepatoprotective effect of *Rhodiola imbricate* rhizome against paracetamol-induced liver toxicity in rats". *Saudi J Bio Sci*, 2014, 21: 409–416.

11.Mitchell JR, Jollow DJ et al. "Acetaminophen induced hepatic necrosis. I. Role of drug metabolism." *J Pharmacol ExpTher,* 1973, 187:185-94.

12. Chen X, Sun CK et al. Protective effect of tea polyphenols against paracetamol‐induced hepatotoxicity in mice is significantly correlated with cytochrome P450 suppression. *World J Gastroenterology,* 2009, 15:1829‐35.

13.Holt MP, Cheng L et al. Identification and characterization of infiltrating macrophages in an acetaminophen –induced liver injury. *J Leukoc Biol*, 2008, 84:1410‐21.

14. Zimmerman HJ, Seeff LB. Enzymes in hepatic disease. In: Goodly EL, editor. Diagnostic Enzymology. Philadelphia, USA: Lea and Febiger, 1970, 1‐38.

15. Campion SN, JohnsonR et al. Hepatic Mrp4 induction following acetaminophen exposure is dependent on Kupffer cell function. *Am J Physiol Gastrointest Liver Physiol,* 2008, 295:294‐304.

16. Ghule BV, Ghante MH et al. Antihyperlipidemic effect of the methanolic extract from *Lagenaria siceraria* stand. fruit in hyperlipidemic rats. *J Ethnopharmacol,* 2009; 124: 333–337.