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Hepatoprotective Activity of *Phylanthus Niruli* Herbs and *Solanum Nigrum* Stem Bark against Paracetamol - Induced Hepatotoxicity

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ABSTRACT

Objective of the present investigation is to evaluate the hepatoprotective activity of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark extracts against paracetamol-induced hepatotoxicity. Materials and methods: Hepatotoxicity was induced in male Wistar rats by oral solution of paracetamol (500mg/kg for 7 days). Aqueous extracts of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark were administered to the experimental rats (300 mg/kg/day, p.o. for 7 days). The hepatoprotective effect of these extracts was evaluated by the assay of liver function biochemical parameters (% of body weight changes, total bilirubin count, total protein, total cholesterol, HDL, SGPT and SGOT level) and histopathological studies of the liver. Results: In Aqueous extracts of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark extract-treated animals, the toxic effect of paracetamol was controlled significantly by restoration of the levels of serum bilirubin, protein and enzymes as compared to the normal and the standard drug silymarin-treated groups. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity. Conclusion: Aqueous extracts of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark possesses significant hepatoprotective activity.

Key words: Hepatoprotective, SGPT, SGOT, HDL, *Solanum nigrum, Phylanthus Niruli*

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INTRODUCTION

Liver, the most versatile but complex internal organ, plays vital role in metabolic activities in human body. It regulates, synthesizes, stores and secretes many important proteins, nutrients, chemical and purifies and clears toxins or unneeded substance from the body [1,2]. Most importantly, the liver is considered to be the center of metabolic transformation of drugs and other toxins entering from the gastrointestinal tract as such the normal or healthy functioning of the liver determines the health status of an individual. Its importance also lies in its impetus in management of internal environment and biochemical conversion of endogenous and exogenous chemical to harmless and excretable compounds. Therefore being a vital organ, its protection has a special status in therapeutics. Prolonged drug therapy, excessive use of the some of the commonly used medicines like paracetamol, diclofenac, nimesulide etc., alcoholism, exposure to certain xenobiotic, pollutants and certain disease state have been reported to affect liver functioning [3].Drug induced liver injury (DILI) is one of the most common causative factor that posses a major clinical and regulatory challenge.[4,5] Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion[1,5,6,7].

Natural products of plant origin containing a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines have hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity [8,9].

Phylanthus Niruli, commonly known as stonebreaker", "gale of the wind", "child pick-a-back", "gulf leaf flower. It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, central and southern India, and China. In India it is usually found as Habit, Leaves, Flowers and Fruit in Maharashtra and Karnataka. The plant has antihepatotoxic, antihypertensive, antiarthritic, antiretriviral, antihyperlipidemic and antihepatitis B medicinal properties [9,10,11,12,13,14,15].

Literature survey indicated that no systematic studies have been carried out on the clinical evaluation of hepatoprotective effect of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark. Hence an attempt was made to screen the aqueous extract of combine form of *Phylanthus Niruli* herbs and *Solanum nigrum* stem bark for hepatoprotective effect against paracetamol induced liver damage in albino rats with comparison to silymarin as standard drug.

MATERIALS AND METHOD

Collection and identification of plant materials

The plant was collected from forest near Bhopal, Madhya Pradesh and authenticated by Scientist Dr. R.B. Goswami (M. Pharm., PhD. Pharmacognosy, Professor & Head, Sagar Institute of Research & Technology – Pharmacy, Bhopal). The plant specimen voucher was deposited in the department, Reference No. 102-121. The plant was shade-dried for 5 days and pulverized using a pestle and mortar. The pulverized part was stored in cellophane bags at room temperature [16,17].

Preparation of extract

The extract was prepared at each time of dosing, to prevent contamination of water. The plant was dried and extracted using decoction process. 5 ml water as solvent was boiled with 0.5 gm of plant to residue 1 ml of the extract. Then extract was filtered and allowed to cool at room temperature.

Animals

This research was carried out in the Departments of Pharmacology, RKDF College of Pharmacy, Bhopal [M. P.] in accordance with the rules governed by CPCSEA (Committee for the purpose of control and supervision of Experiments on Animal), New Delhi, India, between Augusts to November 2010. For this, paracetamol (Suvidhinath Lab, Baroda) and standard drug, silymarin, (Microlabs Ltd., Bengaluru, and Karnataka). A total of 24 adult albino rats of the Wister strain weighing between 120 and 180g were used for hepatoprotective studies. The rats were purchased from the animal house of the Department of Pharmacology, RKDF College of Pharmacy. Following an acclimatization period of 2 weeks, the rats were individually identified by dilute picric acid stain and weighed. The rats were kept in plastic cages under standards. Laboratory conditions at room temperature with 12hrs light/dark cycle with access to standard laboratory diet and drinking water *ad libitum*.

Assessment of Hepatoprotective Effect

In order to assess hepatoprotective action of *Phylanthus Niruli* aqueous extract in albino rats, the rats were divided into the following groups each containing 6 rats (n=6):

Group 1: Control rats: which were fed normal diet and water.

Group 2: Paracetamol treated rats: paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

Group 3: Reference rats: treated with silymarin 100 mg/kg and paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

Group 4: Extract treated rats: received aqueous extract of combine form of *Phylanthus Niruli* and *Solanum nigrum* 300 mg/kg body weight p.o. and paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

After 24 hours of the last treatment, the rats were anaesthetized with anesthetic ether and blood samples from each animal of all groups were collected by retro-orbital plexus puncture in sterilized centrifuge tubes. The blood samples were then allowed to coagulate at 30° C for 45 minutes. Serum portion was separated from each sample by centrifugation at 25000 g at 30°C for 10 minutes and subjected to biochemical investigation to assess liver function on the basis of total bilirubin, SGPT SGOT.

Histopathological Studies

After collecting blood samples, the animals from all groups were sacrificed by cervical dislocation and liver was removed. liver was then cut into small pieces and fixed in 10% neutral formalin solution, embedded in paraffin wax and sectioned at 5µm. Sections were stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was carried out in Deshpandey Research Laboratory, Bhopal [M.P].

Statistical analysis

The results are expressed as means ± standard deviation (S.D.) and values were calculated for each group. A one way analysis of variance (ANOVA) followed by Dunnet's test for significance analysis using Graph Pad Prism software. The minimum level of significance was set of $P<0.05$.

RESULTS AND DISCUSSION

Effect of the aqueous extract on mean organ and body weights

The extract had no significant effect on the body weight of the rats but rats pre-treated with 300mg/kg of the aqueous extract and those administered 500mg/kg of paracetamol showed significant loss in body weight represented in Table 1.

Effect of the aqueous extract on biochemical parameters

Administration of paracetamol had resulted in hepatotoxicity, as evident by significant rise in biochemical parameters. The aqueous extract of combine form of *Phylanthus Niruli* and *Solanum nigrum* at 300 mg/kg body weight p.o. exhibited statisticaly significant reduction in the elevated levels of enzymes selected for the study along with total bilirubin content when compared to paracetamol treated group. A comparable increase in total protein content (6.26 ± 0.15 gm/dl) was observed in extract treated group at a dose of 300 mg/kg body weight p.o. with respect to

paracetamol treated group (4.58 ± 0.17 gm/dl). Treatment with silymarin and aqueous extract of combine form of *Phylanthus Niruli* and *Solanum nigrum* has significantly brought down the elevated levels of SGPT, SGOT, bilirubin, cholesterol and also significantly enhanced the decreased levels of tissue HDL. The results are summarized in Table 2.

Table 1: Shows effect of pretreatment with the aqueous extract of combine form of *Phylanthus Niruli* and *Solanum Nigrum* against paracetamol induced hepatotoxicity on mean liver and body weights.

Groups	Initial body Weight (gm)	Final body weight (gm)	% Body weight difference (gm)
I. Control group (vehicle control)	145.17	150.83	3.99 ± 0.64
II. Toxic group (Paracetamol treated)	154.67	141.17	8.71 ± 0.47
III. Test group (Paracetamol+ Extract treated)	160.33	149.67	6.64 ± 0.6
IV. Standard group (paracetamol +Silymarine treated)	162.17	154.83	4.51 ± 0.17

Table 2: Table shows effect of pretreatment with the aqueous extract of *Phylanthus Niruli* against paracetamol induced hepatotoxicity on biochemical parameters

Groups	Total bilirubin (mg/dl)	Total protein (gm/dl)	Cholesterol (mg/dl)	Hdl (mg/dl)	Sgpt u/l	Sgot u/l
I. Control group (vehicle control)	0.61 ± 0.12	7.48 ± 0.13	107.45 ± 4.4	60.95 ±5.1	75.95 ± 9.5	145.95 ± 8.5
II. Toxic group (Paracetamol treated)	3.77 ± 0.14	4.58 ± 0.17	165.58 ± 9.7	23.91 ±3.8	305.64±8 .6	412.31 ± 4.7
III. Test group (Paracetamol +Extract treated)	1.77 ± 0.14 ***	6.26 ± 0.15 ***	132.24 ± 12***	37.24 ± 5.2**	192.24 ±10 **	290.58 ± 6.4 **
IV. Standard group (paracetamol + Silymarine treated)	1.12 ± 0.084 ***	6.88 ± 0.14 ***	118.91 ± 8.4***	43.41 ± 7.5***	101.74 ± 6.2**	195.08 ± 11 **

Histopathological analysis

In rats of control group, the liver architecture was normal and the cells were arranged radically (Figure. 1). The liver dissected from paracetamol treated rats showed vacuole formation and fatty degeneration. Some of the cells found to have damaged cell walls (Figure. 2). In rats treated with the lower dose of extract i.e. 300 mg/kg body weight p.o. along with paracetamol, the damage was less marked and vacuole formation was observed. The liver cells were observed to be well organized around the central vein along with fat depositions (Figure. 3). The liver cells were

observed to be well organized around the central vein along with fat depositions (Figure. 4). These changes in the liver architecture were coincided with the corresponding changes in the enzyme levels and hence hepatoprotective effect of combine form of *Phylanthus Niruli* and *Solanum nigrum* was confirmed.

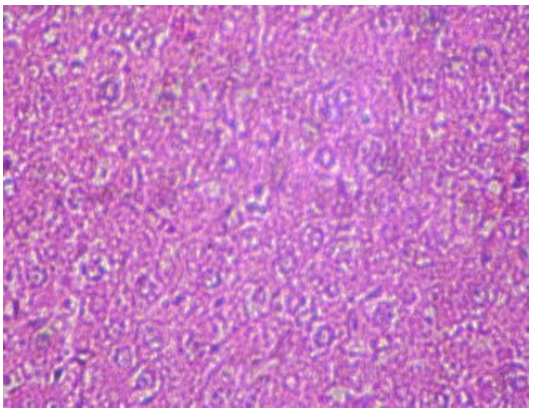

Figure 1: Liver section from control group 1 showing normal liver histo-pathology (Central vein, hepatocytes and portal vein)

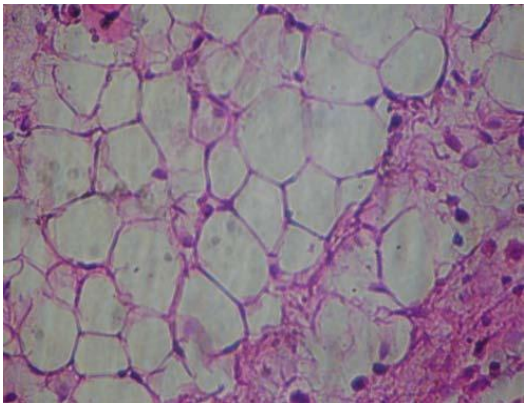

Figure 2: Liver section from paracetamol treated group showing changes of fatty degenerations as well as necrosis of hepatocytes

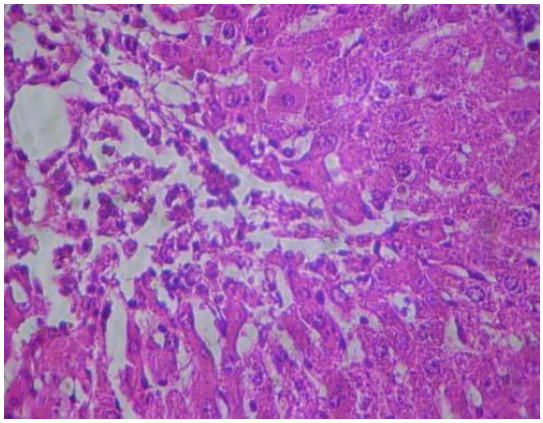

Figure 3: Liver section from combine form of extract treated group (300 mg/kg) showing some necrotic region and regenerative heap.

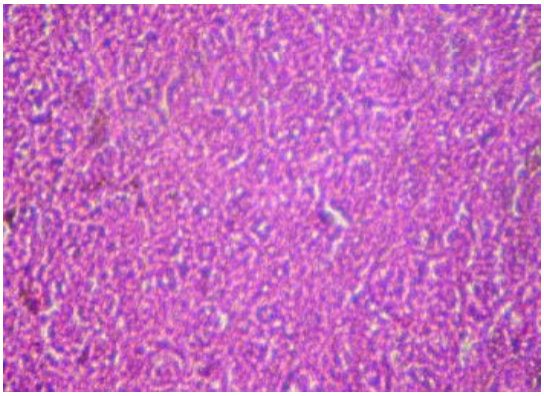

Figure 4: Liver section from silymarin treated group (100 mg/kg) showing central vein and hepatocytes with normal architecture

Paracetamol toxicity, caused by excessive use or overdose of this non-steroidal anti-inflammatory drugs, generally results in liver injury [6,7,18,19]. Toxicity caused by this drug is one of the most common causes of poisoning and acute liver failure worldwide. With progressive disease, signs of liver failure may develop these include low blood sugar, low blood pH, easy bleeding, and hepatic encephalopathy. Some patients spontaneously resolve, although untreated cases may result in death [2,11,20,21]. Since the changes associated with paracetamol induced liver damage are similar to that of acute viral hepatitis, paracetamol mediated hepatotoxicity was chosen as the experimental model.

In this case, damage to the liver or hepatotoxicity results not from paracetamol itself, but from one of its metabolites, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI depletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure[22,23,24,25]. Hepatocellular necrosis leads to elevation of serum marker enzymes, which are released from the liver into blood[26,27]. The increased levels of SGOT, SGPT and total bilirubin (TB) on exposure to paracetamol indicated considerable hepatic injury in present study. The effectiveness of any drug having hepatoprotective action is necessarily dependent on its capability of either reducing the deleterious effect or in maintaining the normal hepatic physiology, which have been altered by a hepatotoxin. In present study, paracetamol-induced liver necrosis was inhibited significantly by combine form of *Phylanthus Niruli* and *Solanum nigrum* extract, which confirmed its protective action against experimentally induced liver damage in rats. SGOT, SGPT and TBL are the most sensitive tests employed in the diagnosis of hepatic disease[28,29,30]. The elevated levels of these parameters due to paracetamol toxicity were significantly reduced by the treatment with aqueous extract of combine form of *Phylanthus Niruli* and *Solanum nigrum*.

CONCLUSION

The aqueous extract of leaves of combine form of *Phylanthus Niruli* and *Solanum nigrum* exhibited potential hepatoprotective activity at dose level of 300 mg/kg weight and this may be due to its rich contents of flavonoids. Hepatoprotective activity of combine form of *Phylanthus Niruli* and *Solanum nigrum* is due to flavonoids is well alkaloidal nature. This finding provides some scientific evidence on traditional use of both the plants.

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