Enhanced Hepatoprotective activity of Piperine Loaded Chitosan Microspheres

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Abstract
The aim of present research was to develop piperine loaded chitosan microspheres to evaluate enhanced hepatoprotective activity in paracetamol induced hepatotoxic mice model. Piperine was extracted from Pippali Piper Longum. Solvent evaporation method was employed to fabricate the microspheres. Optical microscopy demonstrated that the formulation was spherical and had smooth texture with no drug crystals or microsphere aggregation. Formulations containing 2 mg piperine were administered orally to the animal model. In paracetamol induced hepatotoxic mice model, SGOT and SGPT level demonstrated no significant elevation in the blood by the microspheres formulation. The histopathology and enzyme level results suggested that microsphere formulation can passively target hepatoprotective drug to the liver.

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Key words:
Piperine; Microspheres; hepatotoxicity; Chitosan; histopathology.

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INTRODUCTION
Liver is one of the prevalent organs in human body. The major functions of the liver are carbohydrate metabolism, protein metabolism, fat metabolism, detoxification, bile secretion etc. Contemporary medicines have little to offer for the chief site (Liver) for intense metabolism and excretion [1]. Hepatic diseases involve any physiochemical, biological and morphological changes in normal liver functioning. Liver disease is any disturbance of liver function that causes illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those...
functions can cause significant damage to the body. Liver disease is also referred to as hepatic disease [2-3]. Liver disease is a broad term that covers all the potential problems that may occur to cause the liver to fail to perform its designated functions. Usually, more than 75% or three quarters of liver tissue needs to be affected before decrease in function occurs. So dealing with hepatic diseases is predominantly managed by the herbal drugs which have a surprising role in the maintenance, performance [4-5].

Piperine, an alkaloid, is found in the fruits of *Pippali piper nigrum* and *Pippali piper longum*. It is in the forms of white prisms like crystal, which melt at 128°C-219°C. It is almost insoluble in water, but readily soluble in alcohol and ether. It is a very weak base, salts being only formed with mineral acids, and these are dissociated by water [6]. The traditional use includes hepatoprotective, CNS depressant, antipyretic, anti-inflammatory, antibacterial, anticonvulsant and anti-epileptic action [7].

Piperine is principle ingredient in pepper. Several uses of pepper were described since time immemorial in Ayurvedic and Chinese herbs literature. Several direct or indirect evidence of hepatoprotective activity, anticancerous, anticough activity etc has been regularly reported [8]. Thus present study was conducted to evaluate the hepatoprotective activity of the piperine by using paracetamol induced hepatic injury in Wister albino mice.

**MATERIALS AND METHODS**

**Drug and chemicals:** *Pippali piper longum* fruit was purchased from the local market of Sagar, Madhya Pradesh. The authentication of plant was done from Department of Botany of Dr. Sir Hari Singh Gour University Sagar; (MP), Voucher Specimen has been retained in the Department of Dr. Sir Hari Singh Gour University. Paracetamol as bulk drug was obtained as gift sample from Alembic Pharmaceutical Ltd, Baddi (HP), India. The solvent and chemicals used were of analytical grade. Piperine was extracted from Pippali piper longum.

**Preparation of extracts:** *Pippali piper longum* fruit powder was dissolved in 50% methanol and incubated at room temperature (28–30°C) for 16 h. The supernatant collected by centrifugation at 14,000 rpm was dried in vacuum, designated as methnolic extract. This was further fractionated using n-hexane, soluble fraction dried under vacuum and designated as n-hexane extract. The insoluble fraction was further dissolved in chloroform, the supernatant was separated by using a separator funnel. The lower fraction was dried under vacuum, and designated as piperine [9].

**Fabrication of Piperine Microspheres:** The chitosan microspheres were prepared by solvent evaporation method. Chitosan was dissolved in 1.5% (w/v) acetic acid solution, and then piperine was added to the chitosan solution. This drug containing polymer solution was added drop wise in the liquid paraffin containing certain amount of Span 80 used as the emulsifying agent and stirred for 30 minutes at 1500 rpm. The drug content was determined as the 5% of the polymer used. After the formation of w/o emulsion, the cross-linking agent, gluteraldehyde (25% solution in water) was added. Depending on the amount of cross-linking agent the microspheres were cross linked for certain times. After cross-linking, microspheres were centrifuged at 4000 rpm and washed with diethyl ether for several times and finally with acetone. The resulting microspheres were dried at room temperature [10].

**Animals:** Albino mice weighing 25-30 gm of either sex were procured from Veterinary College Mhow (MP). Animals were housed and cages in Sagar Institute of Pharmaceutical Sciences, Sagar (MP) and maintained at 27°C ± 2°C and relative humidity of 30 – 70% with a 12 h dark / light cycle. They were fed with standard mice feed and water was provided. The bedding of the cages was renewed twice a week.
to ensure hygiene and maximum comfort for animals.

**Administration of doses:** Animals were divided into six groups each of six mice. Group I was maintained as a negative control group (no hepatotoxicity). Group II was maintained as positive group (hepatotoxicity induced). Hepatotoxicity was induced by giving paracetamol (3gm/kg body wt. orally) as a single dose on 3rd day. Hepatotoxicity was induced in group II, III, IV, V and VI. Mice of III and IV groups received 2mg and 4mg of plain piperine suspension for 7 days orally. Group II did not receive piperine and acted as untreated group. Group V and VI received 2mg and 4mg piperine containing microspheres for 7 days orally. Suspensions were prepared in distilled water with 0.5% sodium CMC as a suspending agent [11].

**Biochemical parameter estimation:** All the animals were sacrificed on day 8, under light ether anesthesia. The blood samples were collected, separately by carotid bleeding into a sterilized dry centrifuge tube for biochemical investigation i.e. SGOT, SGPT estimation. The clear serum was separated by centrifuging at 2500 rpm, for 10 min [11].

**Histopathological investigation:** The liver from each animal was removed after dissection. The liver was immediately removed and fixed in 10% formalin for 48 hr and were embedded in paraffin, serially sectioned and microscopically examined after staining with hematoxylin and eosin to analyze pathological changes, if any [12].

**Statistical analysis:** The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet’s test followed by Dunnet’s ‘t’ - test. P values <0.05 were considered significant [12]

**RESULTS AND DISCUSSION**

The effects of piperine on Albino mice induced by paracetamol hepatotoxicity were observed in serum enzymes levels like SGPT and SGOT. A significant change in the levels of SGPT and SGOT was observed.

In *vivo* hepatoprotective activity showed that the Group I in which no hepatotoxicity induced shows normal level of SGPT and SGOT. Group II in which the hepatotoxicity was induced shows marked elevation of SGPT and SGOT level. In Group III 2 mg of plain piperine suspension administration shows marginal reversal of elevated SGPT and SGOT level. Group IV wherein, 4 mg of plain piperine suspension was administered also showed marginal reversal of elevated SGOT and SGPT level. However, in Group V the 2mg piperine loaded microspheres shows significant reversal of elevated SGPT and SGOT level as compared to Group III and IV. Moreover, Group VI (treated with 4mg piperine loaded microspheres) exhibited a highest reversal of elevated SGPT and SGOT level as compared to Group III, IV and V which was approximately similar to control normal group Histopathology profile of the control animals showed normal hepatic architecture with discrete hepatic cells well preserved cytoplasm sinusoidal spaces and central vein (A). Disarrangement of normal hepatic cells with intense centrilobular necrosis was observed paracetamol intoxicated liver (B). Moderate accumulation of fatty lobules and cellular necrosis were observed in the animal treated with 2 mg and 4 mg of plain piperine suspension (C and D). However the 2 mg and 4 mg microspheres loaded piperine exhibit a significant liver protection against paracetamol induced liver toxicity, as evidenced by the presence of normal hepatic cords and well defined cytoplasm and absence of necrosis (E and F). The phytoconstituents like piperine possessed exceptional protective action to liver architecture screening its persuasive hepatoprotective activity in mice model.
**Table: 1. In Vivo SGOT and SGPT levels.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT U/ml</th>
<th>SGPT U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive control (No hepatotoxicity)</td>
<td>0.413</td>
<td>0.305</td>
</tr>
<tr>
<td>2</td>
<td>Negative control (hepatotoxicity induced)</td>
<td>1.134*</td>
<td>1.078*</td>
</tr>
<tr>
<td>3</td>
<td>Piperine suspension orally (2mg/kg)</td>
<td>0.976**</td>
<td>0.903**</td>
</tr>
<tr>
<td>4</td>
<td>Piperine suspension orally (4mg/kg)</td>
<td>0.789**</td>
<td>0.767**</td>
</tr>
<tr>
<td>5</td>
<td>Microspheres loaded piperine orally (2mg/kg)</td>
<td>0.581**</td>
<td>0.493**</td>
</tr>
<tr>
<td>6</td>
<td>Microspheres loaded piperine orally (4mg/kg)</td>
<td>0.453**</td>
<td>0.346**</td>
</tr>
</tbody>
</table>

*P<0.001 compared to paracetamol treated group, **P<0.05 compared to control group. Serum enzyme level after 8th day.

**Figure 1:** Histopathology of the liver of various formulation treated mice. (A) Group I (with no hepatotoxicity), (B) Group 2 (hepatotoxicity induced), (C) Group 3, (D) Group 4, (E) Group 5 and (F) Group 6.

**CONCLUSION**

The current study reveals that piperine shows a fine and significant liver protection activity when given to the animals via targeted drug delivery (microspheres). When piperine was given to the experimental animals (Albino mice) then, they exhibit protectant property on liver as compared to toxicant group. So the piperine enhances the hepatoprotective activity and reduces the liver toxicity. The reversal of SGPT and SGOT levels shows the better resilience of targeted drug delivery system (microspheres contains piperine) compare to the conventional dosage of piperine that is 2mg and 4mg of plain piperine.

Targeted drug delivery of piperine (microspheres loaded piperine) shows better bioavailability as the larger dose reaches to the targeted site at liver whereas in case of plain piperine marginal amount of piperine reaches to the targeted site which directly influence the pharmacological or therapeutic action of the drug. Histopathological study reveals that piperine loaded chitosan microspheres reside in the liver and released piperine for a longer period of time, which resulted in higher therapeutic benefit.

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