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# In vitro and invivo evaluation of hepato protection and anti ulcer activities of piperine gastro retentive micropspheres

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## ABSTRACT

**Objective:** In the recent years of research, the interest on herbal medicine is continuously increasing. Piperine is an alkaloid extracted from ripen fruits of *Piper nigrum* and was proved in literature for its hepato protective and anti ulcer properties majorly through anti oxidant capability. **Methods:** In the present investigation gastro retentive, both floating and mucoadhesive microspheres are evaluated for the hepato protection in paracetamol induced model and gastric protection in rats and a comparison was done with conventional microspheres and pure form of piperine. **Results:** The results clearly showed the significant decrease in the serum levels of the marker enzymes in hepato protective study supported by histopathology along with reduced ulcer index in anti ulcer activity. **Conclusion:** This clearly indicates that there is an increase in both activities of floating microspheres, mucoadhesive microspheres when compared with the PP and conventional microspheres.

## 1. Introduction

Liver is the most important organ of human body and it plays an important role detoxification and also the first victim to toxins leading to hepatotoxicity [1]. Most of the liver damages are induced by lipid peroxidation and other oxidative damages caused by toxins. Exogenous factors like smoke, alcohol, stress, fatty food will trigger free radical generation which further leads to mucosal ischemia, excess secretion of hydrochloric acid, pepsin ultimately resulting in ulcers [2]. In India, more than 93 medicinal plants are used in different combination in the preparations of 40 patented herbal formulations [3, 4]. From the herbal source various plants with antioxidant capability as major mechanism along with other mechanisms are used for the hepato and gastric protection [5].

Black pepper is a common spice of intercontinental food and is widely used as carminative, stimulant and also for the treatment of rheumatism, diarrhea, dysentery, cholera and menstrual pain. It is also used in folk medicine for stomach disorders and digestion problems. Piperine from *Piper nigrum* was proved in literature for its hepato protection by reducing the lipid peroxidation [6–8]. Being anti oxidant, black pepper appears to protect gastric mucosa by the stimulation of bioenergetic process and endogenous levels

of co enzyme Q10. The presence of spasmodic (cholinergic) and anti spasmodic (opiod agonist and calcium antagonist) effects of piperine give further support its use in gastro intestinal disorders [9, 10].

From the recent scientific and patent literature, it is evident that there is clear inclination towards the gastroretentive multi unit dosage forms. In gastroretentive dosage forms, while the system retains in gastric environment, the drug is released slowly at desired rate. Thus these are advantageous because of their ability to control the release of the drug at the gastric site without getting cleared from the tract [11].

In our present research, the main objective is to develop gastroretentive floating and mucoadhesive microspheres of piperine and to evaluate their invivo hepato and gastro protection in comparison with conventional microspheres and pure piperine.

## 2. Materials And Methods

### 2.1. Materials

Piperine (98%) from Alfa Aesar, UK and HPMC (Hydroxy Propyl Methyl Cellulose), Carbopal were purchased from SD fine Chemicals Mumbai. Commercially available assay kits for the estimation of serum enzymes were purchased and all other chemicals were of analytical grade.

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## 2.2. Methods

### 2.2.1. Preparation of microspheres by Emulsification solvent evaporation method [12]

Piperine microspheres were prepared by using solvent evaporation method. In brief the procedure includes, Piperine along with polymers were dissolved in acetone. This mixture was then emulsified in light liquid paraffin containing 3% span 80 with continuous stirring at 950rpm at room temperature for 4.5 hours. After evaporation of the acetone the formed microspheres were filtered and washed with petroleum ether to remove the traces of light liquid paraffin. The same method was followed for the preparation of floating microspheres, mucoadhesive microspheres and Conventional microspheres. In floating microspheres, ethyl cellulose, hydroxy propyl methyl cellulose and calcium carbonate were used as polymers. In mucoadhesive microspheres ethyl cellulose, hydroxy propyl methyl cellulose and carbopol were used. In Conventional microspheres, only ethyl cellulose was used. The prepared microspheres were evaluated [13–15] for encapsulation efficiency, particle size, % drug release and buoyancy for floating microspheres, mucoadhesion for mucoadhesive microspheres.

### 2.2.2. In Vivo Hepatoprotective Activity [16]

Adult albino wistar male rats weighing 150–200g were taken from the Technocrats institute of Technology, Bhopal. The animals were maintained in well ventilated room with natural 12 hours day–night cycle and at room temperature in propylene cages. Animals were maintained with standard diet, food and water ad libitum. The protocol was approved by Animal Ethics constituted as per CPCSEA Guidelines (Ref No. TIT/IAEC/831/P'col/2010/1). Paracetamol induced hepatotoxicity in rats was used as a model to determine the hepatoprotective activity. The rats were divided into seven groups with six animals in each group and were given dose schedule as

Control: Animals were not given with either paracetamol or treatment for 14 days.

Group 1: Animals were given with paracetamol 2mg/kg on 6th day per oral and this served as toxic control.

Group 2: Animals were treated with 2 mg/kg, p.o of Slymarin for 1–6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 3: Animals were treated with 300 mg/kg, p.o of Piperine for 1–6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 4: Animals were treated with 300 mg/kg, p.o of Piperine floating formulation for 1–6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 5: Animals were treated with 300 mg/kg, p.o of Piperine mucoadhesive formulation for 1–6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 6: Animals were treated with 300 mg/kg, p.o of Piperine ethyl cellulose microspheres for 1–6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

On the 7th day the animals were sacrificed and various parameters were analyzed. At the end of the experimental period animals were sacrificed by cervical decapitation under mild pentobarbitone anesthesia, blood was collected and the serum was separated by centrifuging at 3,000 rpm for 10 min. The collected serum was used for the assay

of marker enzymes. The enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bile content and total protein content were determined. After draining the blood, liver samples were excised, washed with normal saline and processed separately, for histological observations. The liver was mucoadhesive microspheres immediately removed and fixed in formalin, serially sectioned and microscopically examined after staining with hematoxylin and eosin to analyse pathological changes.

### 2.2.3. In vivo anti ulcer activity [17]

Adult albino wistar male rats weighing 150–200g were taken from the Technocrats institute of Technology, Bhopal. The animals were maintained in well ventilated room with natural 12 hours day–night cycle and at room temperature in propylene cages. Animals were maintained with standard diet, food and water ad libitum. The protocol was approved by Animal Ethics constituted as per CPCSEA Guidelines (Ref No. TIT/IAEC/831/P'ceutics/2010/5).

The dose, 300 mg/kg was selected for the conduct of the experiments were based on preliminary experiments conducted on the pharmacological activity of Black pepper. The route of administration of the aqueous (water) suspension was oral (gastric intubation) in all the experiments. The animals in the test groups were orally administered 1 ml per rat of necrotizing agent (80% ethanol) which is known to produce gastric lesions.

The rats were divided into six groups with six animals in each group and were given dose schedule as

Group I: Animals were not given with either ethanol or treatment for 14 days. This group served as control;

Group II: Animals were given with ranitidine 100mg/kg per oral and this served as standard control.

Group III: Animals were treated with 300 mg/kg, p.o of Piperine

Group IV: Animals were treated with 300 mg/kg, p.o of Piperine floating formulation

Group V: Animals were treated with 300 mg/kg, p.o of Piperine mucoadhesive formulation

Group VI: Animals were treated with 300 mg/kg, p.o of Piperine ethyl cellulose microspheres

Based on the gastric emptying in fasted rats, formulations were given 30 min before the necrotizing agent. Animals were sacrificed under ether anesthesia 1 hr after treatment with ulcerogenic agent. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a magnifier. If there is no ulceration, hyperemia, hemorrhagic spots, 1–5 small ulcers, many small ulcers, many large ulcers and stomach full of ulcers then the ulcer index was given as 0, 0.5, 1, 2, 3, 4, 5 and 6 respectively.

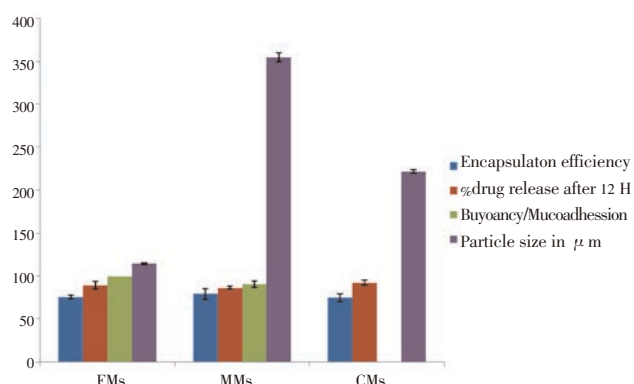
### 2.2.4. Statistical analysis

The data was expressed as mean  $\pm$  SD. Data were analyzed by Dunnett's analysis of variance (ANOVA) to compare all groups against control. Results were considered statistically significant at  $P < 0.001$  and  $P < 0.005$ .

## 3. Results

Microspheres were prepared by solvent evaporation method and the evaluation parameters were given in the figure 1.

Encapsulation efficiency and % drug release were found to be almost equal in all the formulations. Buoyancy was found to be almost 100% for floating microspheres and in the same manner the mucoadhesion was found to be  $91 \pm 1.64$  for mucoadhesive microspheres. The particle size was found to be  $114.5 \pm 1.05 \mu\text{m}$ ,  $354.4 \pm 5.25 \mu\text{m}$  and  $221.6 \pm 2.1 \mu\text{m}$  for floating, mucoadhesive and conventional microspheres respectively.



**Figure 1** Evaluated parameters of the prepared microspheres.

Data source for Figure 1

Parameter	Mean values of various parameters where $n=3 \times 3$		
	FMs	MMs	CMs
Encapsulation efficiency (%)	75.66 ± 0.63	79.21 ± 1.56	75.24 ± 0.94
Mucoadhesion (%)	-	90 ± 1.64	-
Buoyancy in hours	>12	-	-
% Drug release after 12 hours	89.24 ± 1.15	86.34 ± 0.45	92.14 ± 1.02
Particle size in $\mu\text{m}$	114.5 ± 1.05	354.4 ± 5.25	221.6 ± 2.1

From the *in vivo* hepato protective activity given in Table 1, it was found that, the administration of paracetamol caused significant hepato cellular damage which is evident from the increased levels of marker enzymes along with total bile and also there is a decrease in the protein content.

From the *in vivo* antiulcer activity it was found that the degree of ulceration was reduced in all the treatments from 4 to the maximum of 0.75. For control the ulcer index was found to be  $4 \pm 0.894$ . for Ranitidine, pure piperine, floating microspheres, muco adhesive microspheres and conventional microspheres the ulcer indices were,  $0.75 \pm 0.273$  ( $P < 0.001$ ),  $1.5 \pm 0.664$  ( $P < 0.005$ ),  $0.91 \pm 0.204$  ( $P < 0.005$ ),  $2.1 \pm 0.752$  ( $P < 0.001$ ) and  $3.3 \pm 0.516$  ( $P < 0.001$ ) respectively. This

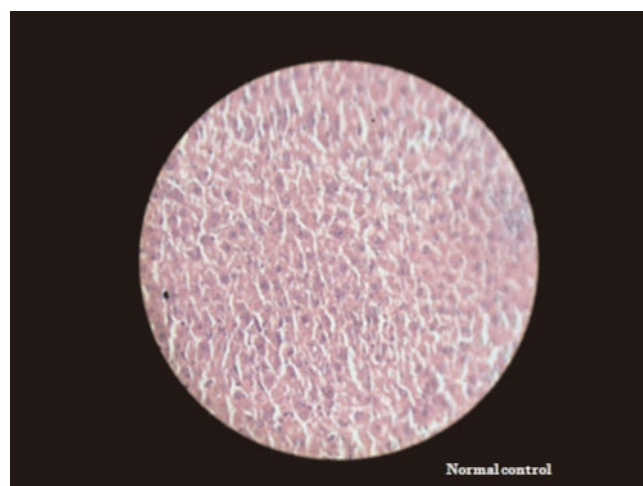
**Table 1**

Serum levels of enzymes in hepato protection of various treatment groups along with control (mean ± SD).

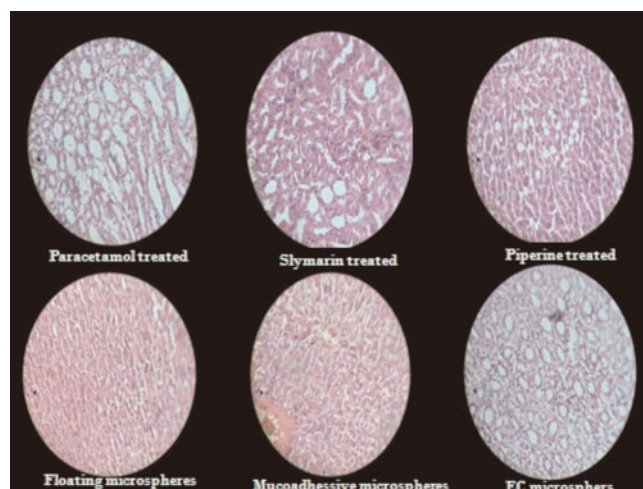
Groups	SGOTU/mL	SGPTU/mL	Total Bile g/dL	ALPU/mL	Proteins g/dL
Control	45.65 ± 5.4	33.47 ± 5.81	0.58 ± 0.014	10.75 ± 2.52	5.8 ± 1.66
1	120.41 ± 6.4*	131.9 ± 4.9**	3.28 ± 0.34**	41.85 ± 1.27**	2.25 ± 0.21**
2	46.40 ± 7.69*	38.83 ± 6.0*	1.01 ± 0.01**	9.18 ± 0.85*	5.76 ± 0.46*
3	115.61 ± 4.3**	128.89 ± 5.75**	2.45 ± 0.09**	31.10 ± 5.3**	1.65 ± 0.33**
4	46.23 ± 4.76*	37.14 ± 5.78*	1.49 ± 0.06**	9.35 ± 1.3*	6.03 ± 0.32*
5	111.61 ± 5.3*	118.69 ± 7.39**	2.3 ± 0.04**	29.75 ± 1.5**	6.08 ± 0.56*
6	117.17 ± 5.7*	129.47 ± 4.26**	2.9 ± 0.04**	41.92 ± 0.81**	3.09 ± 0.04**

\*\* indicates results were considered statistically significant at  $P < 0.001$  and \* at  $P < 0.005$

anti ulcer activity was maximum with the standard drug Ranitidine and then with the floating microspheres.



**Figure 2** Histopathology of rat liver in control group.



**Figure 3** Histopathology of rats in various treatment groups.

#### 4. Discussion

From the above results of microspheres evaluation (Figure 1) there are no significant variations in the evaluated parameters between the prepared microspheres except a slight variation with the mucoadhesive microspheres with more encapsulation efficiency, particle size and less drug release. This may be because of the increased

viscosity of the internal phase with carbopal and HPMC in emulsification process of preparation which was evident even from previous studies [18, 19]. These viscous polymers will form thick boundaries minimizing the drug leaching from the microspheres and also prolong the drug release rate as it has to come out from the viscous matrix of the polymers. The same viscosity will also play important role in formation of large globules while emulsification process during preparation.

The results of hepato protective activity it was found that, all the formulations along with PP and standard drug sylmarin resulted in significant reduction in the enzyme levels than the toxic group indicating the reduced hepato toxicity by paracetamol. The results were further supported by the histopathological examinations. From the Figure 2 and 3, the section of control rat showed the normal hepatic texture but in paracetamol treated had shown cellular necrosis, fatty changes, ballooning, and broadened infiltration of kupffer cells indicating the hepato cellular injury. With mild degree of fatty changes all these damages were significantly prevented in the treatment groups. All the prepared formulations were found to have hepatoprotective activity along with PP. Among the prepared formulations floating microspheres was found to have almost equal activity as that of the sylmarin than the other formulations. Conventional microspheres had shown almost equal activity as that of PP as and less than floating microspheres as they may have the ability to prolong the drug release but they can't retain in the tract. Floating microspheres showed superior activity than mucoadhesive microspheres, this may be because of the lesser particle size ( $\approx 100 \mu\text{m}$ ) than the mucoadhesive microspheres ( $\approx 350 \mu\text{m}$ ) thus increased surface area available for the action.

In vivo antiulcer activity, floating microspheres were able to have good protection against gastric ulcers than other formulations. This may be because of the reason that, the mucoadhesive polymers that were used have swelling tendency in slight alkaline medium (pH-7.0) [20, 21] than in gastric environment and hence adhesion will occur in upper part of small intestine rather in the region of gastric ulcers. The conventional microspheres were inferior to the gastro retentive microspheres as they get rapidly cleared from the tract.

In conclusion, the herbal active principles like Piperine can show better hepatoprotection and antiulcer activities when formulated as novel gastro retentive microspheres rather than the conventional microspheres.

### Conflict of interest statement

We declare that we have no conflict of interest.

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