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Hepatoprotectant Activity of Alcoholic Extract of *Andrographis paniculata* Entrapped in Calcium Alginate Micropellets

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Abstract

Andrographis paniculata, an established hepatoprotectant is widely used in many polyherbal formulations. The main problem with the drug is its bitterness, which causes gastric discomfort, loss of appetite, nausea and vomiting on regurgitation and in turn leads to patient non-compliance. The aim of the present study was to mask the bitterness of *Andrographis paniculata* to encourage its use as a monoherbal hepatoprotectant preparation. The extract of *Andrographis paniculata* (30%w/w of andrographolide) was entrapped into micropellets of calcium alginate for bitter taste masking and the so formed pellets were evaluated for hepatoprotectant activity in paracetamol induced hepatotoxicity in rats. The micropellets containing alcoholic extract of *Andrographis paniculata* equivalent to 22.5mg/kg body weight of the alcoholic extract of *Andrographis paniculata* were given orally to animals for a period of 9 days. The activity observed was compared with alcoholic extract of *Andrographis paniculata*. The bitterless alginate micropellets loaded with alcoholic extract of *Andrographis paniculata* were found to successfully inhibit the paracetamol induced hepatotoxiciy as indicated by a decrease in AST, ALT, ALP and liver weight. The effect observed was supported by histopathological studies. It was concluded that micropellets prepared by inotropic gelation of calcium alignate can be used to mask the bitterness of *Andrographis paniculata* without affecting its activity.

Key Words: *Andrographis paniculata*, Bitter taste masking, Ionotropic gelation, Micropellets, Hepatoprotective, Paracetamol.

1. Introduction

Andrographis paniculata, an established hepatoprotectant is widely used in many polyherbal formulations. Large oral doses of the Herb *Andrographis paniculata* popularly known as “king of bitters” is known to cause gastric discomfort, vomiting and loss of appetite due to its extreme bitter taste [1]. The problem with drugs having high dose is also that, only

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minimum of excepients can be incorporated in their formulation which are again insufficient to mask the bitterness of andrographolide. So converting the drug itself into bitterless micropellets that can be directly filled into a capsule or compressed into tablets can solve a major formulation, patient compliance and manufacturing problem with *Andrographis paniculata*. Further, avoiding dose dumping of the drug extract in the stomach can help in solving the problems of nausea on regurgitation. If required these pellets can be further coated with release modifying polymers.

The delivery system used in this study primarily attempts to incorporate *Andrographis paniculata* in a taste masked matrix system and present it in the form of a mono-herbal therapy to maximize its potential as a hepatoprotectant. So the aim of the present study was to evaluate the so formed calcium alginate micropellets of *Andrographis paniculata* for hepatoprotectant activity.

2. Materials and Methods

2.1 Materials

Alcoholic extract of *Andrographis paniculata* containing 30%w/w of andrographolide (Natural Remedies Pvt Ltd, Bangalore India), Sodium alginate and Calcium chloride (Loba Chemie Pvt. Ltd, Mumbai, India), Aspartate amino Transferase (AST), Alanine amino Transferase (ALT) and Alkaline Phosphatase estimation kits (Span Diagnostic Ltd, Surat, India) and all other reagent were of analytical grade.

2.2 Experimental animals used

Male adult albino rats of Wistar strain weighing between 240-280 g were selected for the experiment. They were divided into 4 groups consisting of 6 animals each. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard laboratory conditions in an animal house approved by the committee with the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).The animals had free access to commercial pellet diet and water *ad libitum*.

2.3 Preparation of Andrographolide loaded calcium alginate micropellets [2].

A 2.5% w/v solution of sodium alginate was prepared by dissolving sodium alginate in deionised water under continuous stirring. This solution was sonicated for 10 minutes and the drug (ratio 2:1::drug:polymer) was dispersed in small quantity of water before being incorporated into the alginate solution. This dispersion was added drop wise into a 2%w/v solution of calcium chloride using a hypodermic syringe with a flat tip needle (20 G). The calcium chloride solution was simultaneously stirred at a speed of 50 rpm using a magnetic stirrer. The drug-loaded pellets which were formed immediately on contact with the calcium chloride solution were allowed to cure for 30 minutes in the calcium chloride solution to complete the gelation reaction. Then the so formed micropellets were filtered and washed repeatedly with deionised water in increments and dried using hot air oven at 60°C for 5 hrs.

Evaluation of hepatoprotective activity

The animals were divided into four groups consisting of six animals each and they were given the following treatments.

Group 1 : Normal Group: Animals received distilled water (2 ml/kg) p.o for 10 days.

Group 2 : Paracetamol Control Group: Animals received distilled water p.o for 9 days.

Group 3 : Drug Extract Group: Animals received 25.2 mg/kg of alcoholic

extract of *Andrographis paniculata* (containing 30% w/w of andrographolide) for 9 days.

Group 4 : Test Group: Animals received Andrographolide-loaded micropellets equivalent to 25.2mg/kg body weight of alcoholic extract (30% w/w) for 9 days.

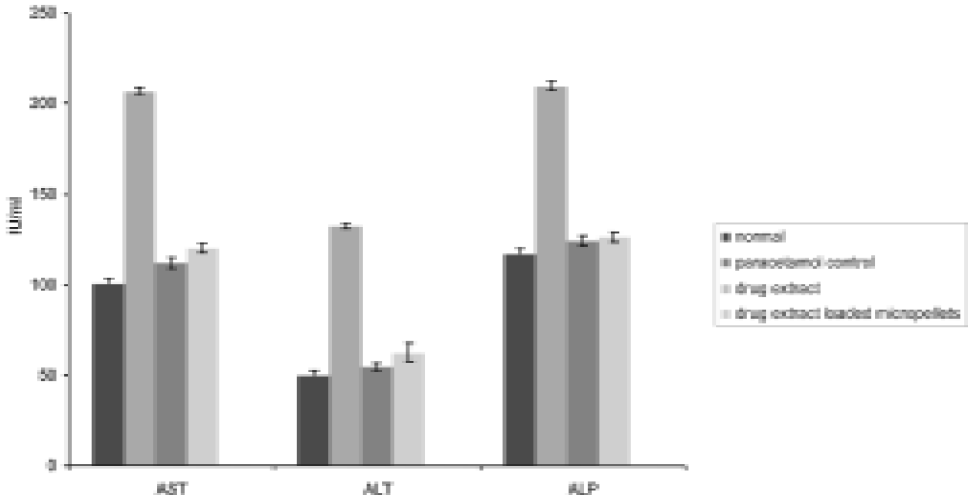
Food was withdrawn 12 hrs prior to paracetamol administration to aggravate the acute liver toxicity in animals of group 2, 3 and 4. Single dose of Paracetamol (1gm/kg body weight, p.o) diluted with sucrose solution (40%w/v) was administered on the 10th day in three divided doses to group 2, 3 and 4 and sacrificed 48 hrs after administration of paracetamol [3]. Blood samples were collected from the abdominal aorta and serum was used for determination of the AST, ALT and ALP levels. The liver was isolated and rinsed with cold phosphate buffer. The absolute weight of liver for the different groups of animals was recorded to the nearest milligram [4]. This liver was preserved in 10% formaldehyde solution for histopathological studies.

2.4 Statistical analysis

One way Analysis of variance (ANOVA) followed by Tukey multiple comparison test was used to estimate variations in the data obtained using Graph Pad Instat Software. $P<0.05$ was considered significant.

3. Results

The serum levels of AST, ALT and Alkaline phosphatase after 48 hrs of paracetamol administration were significantly increased ($p< 0.001$). Pretreatment with the micropellets containing alcoholic extract of *Andrographis paniculata* (22.5mg/k.g body weight) reduced the levels of the biochemical markers significantly when compared to the paracetamol control ($p<0.001$). Similar results were noticed with alcoholic extract of *Andrographis paniculata* (Fig 1).



All values are mean ± SEM, n=6, +++P<0.001 compared to normal, ***P<0.001 compared to paracetamol control

Fig 1: Effect of alcoholic extract of *Andrographis paniculata* (30%w/w) and Micropellets loaded with the alcoholic extract of *Andrographis paniculata* (30%w/w) on serum AST, ALT and ALP in paracetamol induced Liver damage in rats.

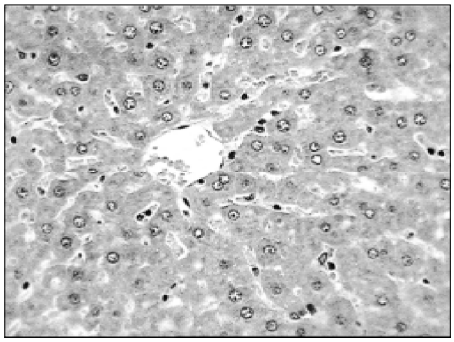


Fig. 2: Photomicrographs showing normal liver histopathology

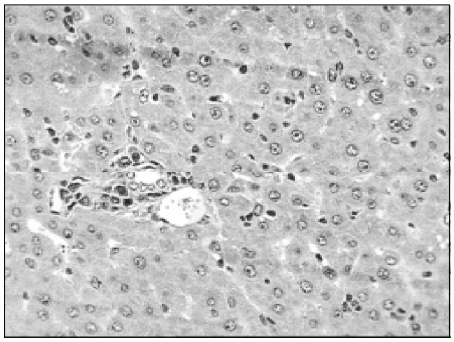


Fig. 3: Photomicrographs showing paracetamol induced liver damage

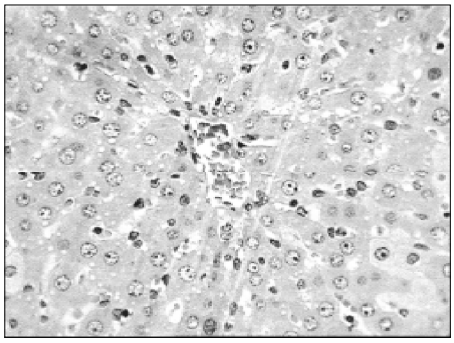


Fig. 4: Photomicrographs showing effect of alcoholic extract of *Andrographis paniculata* on paracetamol induced liver damage.

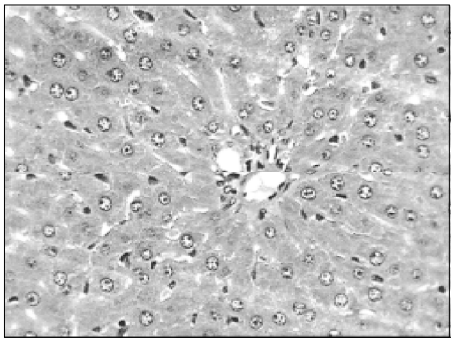


Fig. 5: Photomicrographs showing effect of micropellets loaded with the alcoholic extract of *Andrographis paniculata* (30%w/w) on paracetamol induced liver damage

Table 1. Effect of alcoholic extract of *Andrographis paniculata* (30%w/w) and micropellets loaded with extract of *Andrographis paniculata* on changes in Liver weight in Paracetamol induced Liver damage in rats.

Sl No.	Treatment	Dose p.o	Liver weight /100gm of body weight
1	Normal (Vehicle)	2.5 ml/kg	1.975 ± 0.2284
2	Paracetamol treated Control (Vehicle)	2.5 ml/kg	3.59 ± 0.26+++
3	Alcoholic Extract of *Andrographis paniculata* (30%w/w of andrographolide)	100 mg/kg	2.36 ± 0.11**
4	Micropellets loaded with alcoholic extract of *Andrographis paniculata*	=22.5mg/kg of alcoholic extract (30%w/w of andrographolide)	2.66 ± 0.18*

+++ P 0.001 when compared with normal control. ** P 0.01 compared to paracetamol treated control.
* P 0.05 compared to paracetamol treated control

The weight of the liver of the animals pretreated with the extract loaded micropellets was significantly lower when compared to the paracetamol control ($p<0.05$). The alcoholic extract of *Andrographis paniculata* showed better protection compared to the micropellets (Table 1).

Further, histopathological examination of the liver treated with paracetamol showed damage to the hepatic architecture, ballooning necrosis with lobular disarray, mild fatty changes, kupfer cell hyperplasia, crowding of central vein, central and peripheral portal inflammation, bridging necrosis and regeneration of cells (Fig 3). Sections of liver treated with the alcoholic extract of *Andrographis paniculata* showed architectivity similar to that of the normal liver (Fig 2), however, mild kupfer cell hyperplasia was noticed (Fig 4). The liver from animals treated with the micropellets were similar to normal animals but for the mild ballooning necrosis observed (Fig 5).

4. Discussion

Paracetamol is known to induce liver injury through action of its toxic metabolite, N-acetyl-p-benzoquinoneimine produced by the action of Cytochrome P-450. The metabolite reacts with reduced glutathione [GSH] to yield non-toxic 3-GS-yl – paracetamol. Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to cell-death [5, 6]. This damage induced in the liver is accompanied by the increase in the levels of the serum marker enzymes [7]. Paracetamol is also known to increase the weight of the liver due to blockage of secretion of hepatic triglycerides into the plasma [8]. The *Andrographis paniculata* loaded micropellets and the alcoholic extract of the drug showed hepatoprotectant action by significantly attenuating the increased levels of serum enzymes; AST, ALT and alkaline phosphatase in rats intoxicated by paracetamol. The increase in liver weight was also controlled in the groups treated with the micropellets and alcoholic extract of *Andrographis paniculata*. The extract of *Andrographis paniculata* entrapped in alginate micropellets was as effective as the alcoholic extract of the drug. The results obtained in the present study substantiates the results of the dissolution studies carried out earlier in our lab, wherein the micropellets demonstrated optimum release of drug in pH 7.4 in a controlled manner (unpublished data). Further FTIR, MTDSC and FTRaman studies demonstrated that the integrity of the drug was maintained in the micropellets with no interaction whatsoever with the polymer used (unpublished data). Micropelletisation using ionotropic gelation of sodium alginate has been previously used for preparing barrier systems [9] and for local or controlled delivery of water insoluble drugs [10]. It has also been used for site specific delivery of drugs into the G.I.T. In the present study, we have exploited this technology to successfully mask the bitterness of *Andrographis paniculata* and yet retain its hepatoprotective activity in order to administer large doses of the drug in a patient compliant form. The use of an unsophisticated equipment, aqueous solvent system coupled with a cost-effective natural polymer provides immense potential for formulation of extracts of *Andrographis paniculata* into free flowing micropellets that can be used as such requiring no further addition of excepients.

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