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Formulation, Excipient Properties and Pharmacological Activities of Catechin Liposomes

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ABSTRACT

Liposomes containing catechin were prepared using soya lecithin by thin film hydration technique. The entrapped vesicles were characterized for their shape, size, drug entrapment efficiency and *in vitro* release rate. Excipient properties were observed using curcuminoids. The liposomal formulation was able to protect the curcuminoid solution from degradation to some extent. The resulting formulation was evaluated for antioxidant activity *in vitro* and screened for hepatoprotective activity against carbon tetrachloride induced toxicity and antidiabetic activity against alloxan induced diabetes *in vivo*. The present study revealed that catechin liposomes showed good antioxidant property similar to ascorbic acid but failed to show hepatoprotective and antihyperglycemic activity with the selected doses.

KEY WORDS: Catechin, Liposomes, Hepatoprotective activity, Antidiabetic activity.

INTRODUCTION

Catechins, a group of similarly structured polyphenolic phytochemicals of chemical and medicinal value (1) come under pharmacognostic classification as 'flavonoids' are poorly absorbed through the gastrointestinal and the examples include catechin, epicatechin, epigallocatechin, gallocatechin, epigallocatechin gallate (EGCG) (2,3,4). Heating catechins past their point of decomposition release pyrocatechol, which explains the common origin of the names of these compounds (2). They demonstrated very strong antioxidant activity both *in vitro* and *in vivo* conditions. Our current study deals with investigations on catechin, one of the flavonoids belonging to catechins family of phytochemicals. Catechin was first isolated from plant extract catechu, Acacia catechu – Leguminosae from which it derives its name. Catechin and epicatechin are epimers with (-)-epicatechin and (+)-catechin are optical isomers found in nature. Naturally, catechin exists as a mixture of (-)-epicatechin and (+)-catechin (3). As is similarly true with other flavonoids, catechin is poorly absorbed across the GIT because it has multiple ring molecules that are too large to be absorbed by simple diffusion; it typically has poor miscibility with oils and other lipids which limit their ability to pass across the lipid rich outer membranes of enterocytes of small intestine and catechin consists of two benzene rings (A and B) and a pyran ring (called C – ring), which might be the cause for poor oral absorption (4).

Catechin has demonstrated several pharmacological benefits. H. J. Reimann (1977) in his study reported that catechin prevented the formation of acute gastric lesions by 80% by series of experiments lasting half a year. As the drug has low toxicity in man, it was recommended for clinical trials (5).

A. I. Rivkind (1983) experimentally proved that catechin at a dose of 36 or 72 mg per rat administered intraperitoneally substantially inhibited adhesion formation which was induced by gentle scraping (6). M. J. Weyant (2001) reported the efficacy of catechin to inhibit intestinal tumor formation and suppression of focal adhesion kinase activation in the Min/+ mouse. Administration of (+)-catechin in an AIN – 76A diet at doses of 0.1 and 1% decreased the intestinal tumor number by 75 and 71% respectively (7). S.Kalender (2002) investigated the effect of catechin and vitamin E on reducing toxic effects of Idarubicin (anthracycline antibiotic used in treating acute leukemia) in rats. It was showed that catechin significantly reduced Idarubicin induced carditoxicity in rats (1). F. Takano (2004) studied the protective effect of (+)-catechin against 5-fluorouracil induced myelosuppression in mice (8). Intraperitoneally injected (+)-catechin (1 and 10mg/kg per day) accelerated the recovery of number of white blood cells and platelets against the hematotoxicity produced by 5-fluorouracil in mice. These findings suggested that (+)-catechin selectively enhances the recovery of population of granulocytes

reduced by 5- flurouracil in mice. Similarly, catechin demonstrated benefit in chronic fatigue syndrome (9), prevention of tamoxifen induced oxidative stress and biochemical perturbations in mice (10), hepatoprotection (11,12).

The first aim of this study is to investigate the use of catechin in hepataoprotection with the assistance of liposomes as the delivery systems. Thus, this present study was undertaken by putting forth all the previous reports on efficacy of catechin as hepatoprotective agent. Liposomes are capable of delivering hepatoprotective agents to the liver to increase local concentration of agent and to reduce adverse effects to achieve maximal therapeutic efficiency. Thus, liposomal formulation encapsulating catechin was investigated for hepatoprotective action. The second aim is to investigate the same dosage form in diabetes. Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular disease (13). There were some reports, which stated that catechin could be used for the treatment of Diabetes mellitus (14,15,16). Some other reports stated that catechin could not relieve the symptoms of diabetes mellitus and hence they reported that it could not be used for treating diabetes mellitus (17,18). From the above reports, the present study has been undertaken to investigate whether catechin is antidiabetic or not. Liposomal delivery of catechin could lead to higher tissue levels of the active at the target site, in this case, pancreas thereby leading to solving discrepancies already published with regard to catechin as an antidiabetic agent.

MATERIALS

Catechin was procured from Yucca enterprises, Mumbai. Alloxan monohydrate was purchased from sigma Aldrich, Germany. SGOT and SGPT kits were procured from Qualigen Chemicals, Mumbai. Soya lecithin, chloroform, sodium phosphate, ammonium molybdate, and all other chemicals used in this study were procured from local suppliers and they were all of analytical grade. Albino rats were purchased from Mahaveer Enterprises, Hyderabad

Methods

Preparation of Catechin liposomes intended to be administered by IV route

Liposomes are made up of phospholipids present on the cell membranes (19). Several types of liposomes including multilamellar vesicles, small unilamellar vesicles, etc. exist. Multilamellar vesicular liposomes encapsulating catechin were prepared by thin film hydration technique (20). The formulation contains: Catechin (30 mg) and soya lecithin (50 mg) in 10 ml of normal saline. As a first step, thin film of the lipid and drug was prepared on the bottom of the round bottomed flask. Phospholipid i.e., soya lecithin, catechin were weighed as per the formula and dissolved in chloroform in a round bottomed flask of rota evaporator. 5 ml of ethanol was added to dissolve catechin. The solvent was then evaporated in a rota evaporator under reduced pressure at a temp of 60 $\pm$ 5^{o}C and flask was rotated at 90 rpm to create a thin layer of lipidic film which was deposited on inner wall of round bottom flask. MLVs were prepared by hydrating the thin film. 10 ml of normal saline was added to the thin layer of round bottom flask to hydrate the layer. RB flask was rotated at 180 rpm at a temp of 70 $\pm$ 5^{o}C for about an hour to form the suspension of liposomes. The liposomes obtained consist of multilamellar vesicles (MLVs). In the next step, SUVs of catechin that can be injected by intravenous route were prepared. SUVs were produced by sonicating MLVs in a bath sonicator. Sonication of MLV dispersion was accomplished by placing a test tube containing the liposome suspension in a bath sonicator and sonicated for 10-15 min above Tc of lipid. A transparent solution *was obtained due to break down of MLVs to SUVs.*

Liposome characterization

The prepared liposomes were characterized for particle morphology, size and drug release. Ordinary microscope was used for particle size measurement. A drop of liposomal formulation was mounted on a slide and placed on a mechanical stage. The microscope eyepiece was fitted with micrometer by which the size of particles can be estimated. The field was focused on the slide and sizes of 50 particles were measured and the average was taken to determine the range of particles.

To determine in vitro drug release, commercially available dialysis membrane was used which was soaked in distilled water at 50^{o}C for 30 min. The membrane was taken out and fixed in balloon shape to the tip of test tube containing 1 ml of liposomal formulation. The test tube was inverted and introduced in to a beaker containing stirrer to maintain vortex in the medium. Samples were withdrawn at intervals of one hour for six hours and then 24, 48, 72 hrs., upto 7 days. Medium was replaced with water each time samples were withdrawn. The samples were

measured for absorbance using UV spectrophotometer at 280 nm.

In vitro antioxidant activity of the catechin at the end of fabrication of liposomes

Fabrication may lead to the changes in the catechin used. Additionally, the catechin might decompose during release. To test the activity at these stages antioxidant activity was measured. Evaluation of total antioxidant capacity of catechin liposomes was done by using phosphomolybdenum method (21). An aliquot of 0.1 ml of sample solution of various concentrations was added with 1 ml reagent solution. (0.6 M sulfuric acid, 26 mm sodium phosphate and 4 mm ammonium molybdate) for the blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and incubated in boiling water at 95^{o}C for 90 min. After the samples had cooled to room temperature, the absorbance of aqueous solution was measured at 695 nm by spectrophotometer against a blank. Antioxidant capacity was expressed as equivalents of ascorbic acid. Similarly total antioxidant capacity of catechin was also measured.

Excipient properties of catechin liposomes

Catechin is a powerful antioxidant. Curcuminoids are unstable in light and it also get degrade at P^H of stomach, and intestine. This is a novel approach to investigate whether catechin by its antioxidant properties can prevent degradation of curcuminoids at different P^Hs.

0.5 mg of Curcuminoids were dissolved in 5 ml of methanol. 5% catechin liposomes were prepared in methanol. Phosphate buffers of P^H 1, 5 and 8 were prepared. 2 ml of curcuminoid solution was added to 6 ml of phosphate buffer P^H 1 to which 2 ml of catechin liposomes were added. Similarly solutions were prepared with P^H 5 and 8. Test solutions were also prepared excluding catechin liposomes with different buffer solutions and also solutions were prepared excluding curcuminoid solution. Absorbances of all these solutions were measured at 425 nm using UV-Visible spectrophotometer after 12 hrs.

Pharmacological Activities

Hepatoprotective activity

Experimental animals were divided into three groups, each group containing three rats. Male rats weighing 200-250 gm were used in the present study. Group I was normal control group. Group II and III received a single dose of mixture of carbontetrachloride and olive oil (0.5 ml in 20 ml) intraperitoneally on 1 st day of study. Group II served as carbontetrachloride induction control. After 6 hrs of administration of carbontetrachloride, Group III received catechin liposomes (SUVs 2 mg / each rat) intravenously. On 4 th day, blood was collected from the reto orbital sinus of rats from all three groups. Blood was collected and allowed to clot for 30 min., serum was separated and centrifuged at 3000 rpm for 5 min and the separated serum was used for estimation of biochemical parameters i.e., SGOT and SGPT levels.

Antidiabetic activity

Animals were divided into three groups containing three rats in each group. Male rats weighing 200-250 gm were selected. The animals were fasted for 16 hrs. Alloxan monohydrate at a dose of 120 mg/kg body weight was dissolved in normal saline and administered intraperitoneally to all the II and III groups. After 48 hrs, glucose levels were measured by using glucometer with C 20 one touch glucose strips. Rats with glucose levels greater than 140 mg/dl were selected for the study. Group I served as normal control group. Group II was alloxan induction control. Group III was administered with catechin liposomes (SUVs 2 mg/each rat) intravenously. Glucose levels were measured on 1st, 2nd, 3rd day.

RESULTS AND DISCUSSION

Liposomes encapsulating catechin were prepared using soya lecithin, catechin, chloroform, ethanol and normal saline by thin film hydration technique. Multilamellar vesicles (MLVs) were formed which on sonication formed small uni lamellar vesicles (SUVs). Optical microscopy showed liposomes (MLVs) produced were having spherical shape. Drug content in the formulation was estimated by UV spectrophotometer at 280 nm. Percent loading efficiency was found to be 80%. Particle size analysis of the liposome formulation was done by optical microscopy. The average particle size was found to be in the range of 1.2 μ. A standard graph for catechin was plotted between absorbances versus concentration (Figure 1), which was subsequently used to obtain the concentration of unknown samples. To assess the rate of release of drug from the formulation, in vitro release studies were performed. The drug release from the formulation was observed for seven days. Samples were withdrawn at intervals of 1, 2, 3, 4, 5, 6, 24, 48….. for 7 days and absorbances were measured by UV spectrophotometer at 280 nm. *In vitro* release studies were performed for both MLVs (Figure 2) and SUVs (Figure 3). In the first two hours of release, the drug, which was not encapsulated, released fast, so release is high initially and decreased, later the encapsulated drug released gradually, so a gradual increase was seen in the

Table 1 Excipient Properties of Catechin Liposomes

P^H	Curcuminoid solution +buffer	Catechin liposomes+buffer	Curcuminoid+buffer+Catechin liposome solutions
1	0.005	0.010	1.275
5	0.276	0.057	1.514
8	0.059	0.125	1.394

Table 2 Total Antioxidant Activity of Catechin

Concentration (µg/ml)	Absorbance at 695 nm
5	0.069
10	0.073
15	0.112
20	0.132
25	0.145
30	0.168

*Compared with Ascorbic acid, Catechin has similar antioxidant capacity.

Table 3 Total Antioxidant Activity of Catechin Liposomal Formulation

Concentration (µg/ml)	Absorbance at 695 nm
30 mg drug	2.781

*Compared with Ascorbic acid 35 mg = 2.720

Table 4 Hepatoprotective Action of Catechin Liposomes

Groups	Biochemical parameters	
	SGOT (IU/L)	SGPT (IU/L)
Group I Control	0.348 ± 0.018	0.043 ± 0.018
Group II CCl4 treated	0.916 ± 0.923	0.088 ± 0.030
Group III CCl4 + Catechin liposomes	0.334 ± 0.176	0.109 ± 0.032

Results are reported as mean ± SD

Table 5 Antidiabetic Activity of Catechin Liposomes

Groups	blood glucose levels (mg/dl)	After administration of drug (mg/dl)	
	1st day	2nd day	3rd day
Group I Control	72.66 ± 2.51	70.66 ± 1.15	80 ± 10
Group II Alloxan treated	230.66 ± 7.63	216.66 ± 4.16	233.66 ± 7.09
Group III Alloxan + Catechin liposomes	201.66 ± 1.15	216.66 ± 4.16	259 ± 4.58

Results are reported as mean ± SD

release. Release was good for 3 days and gradually decreased. Catechin liposomes have shown good activity in protection of curcuminoid solution at P^H 1, 5 and 8. Buffer solution could not prevent it from degradation but in the presence of catechin liposomes, oxidation was dramatically reduced (Table 1).

Both catechin and catechin liposomes showed the antioxidant capacity, which is comparable to ascorbic acid (Table 2 and 3). Antioxidant activity can be attributed to the flavonoids present in the catechin.

Catechin liposomes were screened for hepatoprotective action in albino rats. Induction of carbontetrachloride increased the SGOT and SGPT levels indicating the liver injury. Catechin liposomes showed a decrease in SGOT levels similar to control group. No change was observed in SGPT levels even after administration of catechin liposomes. The present study is to treat hepatotoxicity at a very lower dose formulating it as liposomes. Catechin liposomes at a dose of 2 mg/each rat decreased the SGOT levels but

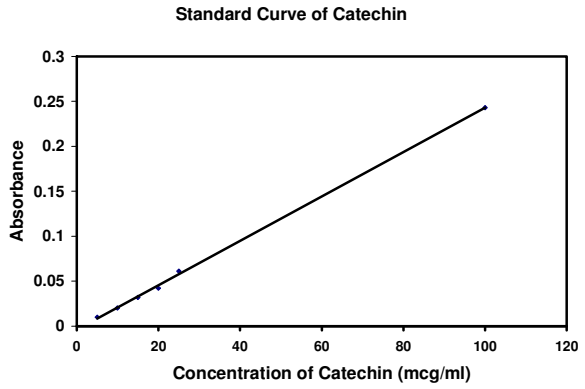

Figure 1

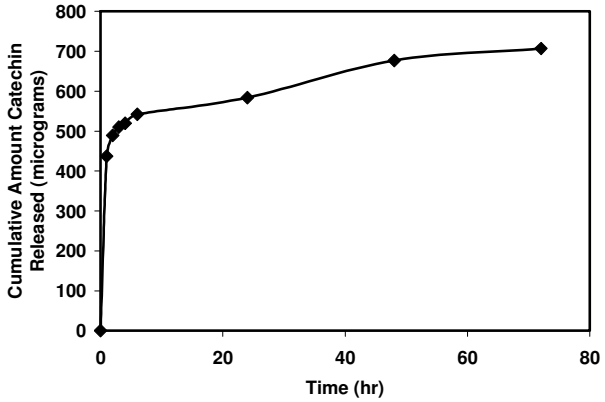

Figure 2 Drug Release Studies with MLVs

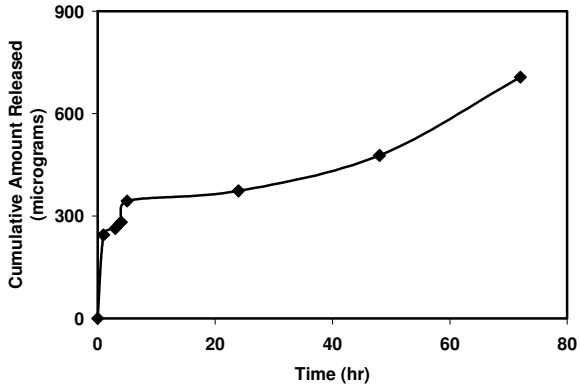

Figure 3 Drug Release Studies with SUVs

failed to decrease SGPT levels. So further investigation is needed by increasing the dose (Table 4).

Antidiabetic activity of catechin liposomes was done by using alloxan induced diabetic rats. Alloxan monohydrate (120 mg/kg) produced hyperglycemia in rats. Catechin liposomes at a dose of 2 mg/each rat could not prevent the hyperglycemia caused by alloxan and so there was no decrease observed in blood glucose levels even after administration of catechin liposomes (Table 5).

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