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Research Article

DOSE-DEPENDENT HEPATOPROTECTIVE ACTION OF ALGINATE BIOPOLYMER ENCAPSULATED CATECHIN NANOPARTICLES

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ABSTRACT: Catechins are the predominant form of flavonoids present in plants and have high antioxidant capacity, but their usage is limited in clinical practice due to low bioavailability. Nano formulation of catechin was prepared by using sodium alginate polymer to address the bioavailability problem. Nanoparticles were synthesized by the co-precipitation method. In vivo antioxidant property of alginate-coated catechin nanoparticles was assessed against carbon tetrachloride-induced hepatopathy in a rat model. Rats were randomly divided into ten groups, with six rats (n = 6) in each group. Carbon tetrachloride @ 4 ml/Kg body weight was used to cause subacute hepatotoxicity in rats; Group I (healthy control), Group II (disease control), Group III (blank catechin), Group IV (blank alginate), Group V (Silymarin), Group VI and Group VII (Alginate coated catechin nanoparticles, two different dose intervals), Group VIII, Group IX, Group X (Alginate coated catechin nanoparticles, Three different doses). Oxidative stress assay, histopathological examination, and May-Grünwald staining were done on the 28th day of the trial. Haemato-biochemical parameters and oxidative stress markers on the 28th day in the rats of Group VI showed significant improvement as compared to other groups. Significant improvement in the healing pattern of gross tissues of the liver was observed in Group VI. May-Grünwald Giemsa staining depicted prominent regenerative changes in liver cells in Group VI. The study concluded that the alginate coating of catechin improves the bioavailability and depicted better regeneration of hepatocytes.

Keywords: Alginate, Antioxidant, Catechin, Liver, Nano formulation.

INTRODUCTION

The liver is the primary site of detoxification in the body and acts as the first line of defense against infectious, toxic, or carcinogenic agents coming from the gut [1]. Many acute and chronic clinical diseases, metabolic disorders, degenerative processes, vascular injuries, autoimmune diseases, and even physical trauma, can result in hepatobiliary dysfunctions (HBD). There is growing evidence that oxidative stress has a role in the development and course of hepatic damage [2]. Due to their bioavailability, therapeutic concentration on the target organ, and sometimes even negative side effects, medications used to treat hepatic damage have limited therapeutic benefits. Therefore, investigating new and different methods for liver regeneration is crucial. Antioxidant-rich natural compounds have garnered a lot of interest as possible useful components for hepatopathy. Strong antioxidants including piperine, quercetin, curcumin, catechin, glycyrrhizin, etc. are found in many plant-based

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compounds and may aid in the healing of hepatic injury when they are properly bioavailable at the target organ [3]. The most common type of flavonoid found in plants is called catechin, which has gained special attention because of its comparatively high antioxidant capacity in the biological system [4]. In comparison to the corresponding crude drug preparations, nanoformulation offers numerous benefits, including improved medicine absorption, decreased dosages, and increased solubility of compounds [5]. Alginate's biodegradability, biocompatibility, and mucoadhesion have made it useful in a variety of biomedical applications, such as drug administration systems [6]. The potential of using nanoparticles in the administration of medications was demonstrated by the preparation and evaluation of a sodium alginate-coated catechin nanoparticulate carrier system using an experimental rat hepatic damage model.

MATERIALS AND METHOD

Experimental design

Details of the experimental design for the therapeutic trial on rats are shown in Table 1.

Preparation of the alginate polymer-coated catechin nanoparticles

Catechin-loaded alginate polymeric nanoparticles were prepared by the facile co-precipitation method [7] with minor modifications. The particle size of the prepared formulation was 38.24 ± 4.71 nm with an encapsulation efficiency of 61.1% [8].

Ameliorative effect of alginate polymer coated catechin nanoparticle against carbon tetra chloride 4 induced hepatopathy in rats.

Sixty (60) male albino Wistar rats, weighing 90-120 gm were kept in an experimental animal shed of the Division of Medicine under regular management and feeding guidelines by IAEC standards. Rats were randomly divided into ten groups having six rats (n=6) in each group. Subacute hepatotoxicity was induced in rats by administering carbon tetrachloride with olive oil (1:1, v/v) @ 4 ml/ kg body weight as oral lavage twice a week for four weeks [9]. Following completion of the trial, rats were euthanized as per IAEC norms and 2-3 ml blood samples from anesthetized rats were collected by cardiac puncture using heparinized sterilized syringes for hematological study. Liver samples were collected for the study of oxidative stress parameters and histopathological studies.

Oxidative stress assay in the liver tissue

500 mg of liver tissue was placed in 5 ml of ice-cold PBS (pH 7.4) to measure the oxidative stress indicators. For the determination of (reduced glutathione) GSH, an additional 200 mg of liver tissue was collected and dissolved in 2 ml of 0.02 M EDTA in distilled water. The 10% liver homogenate was made using a homogenizer in an ice-cold environment. It was then centrifuged for 10 minutes at 3000 rpm, and the supernatant was kept at –20°C until it was tested.

Lipid peroxidation (LPO) assay

The lipid peroxides level was determined in tissue homogenate following the method suggested by [11]. Using 1.56×10-5 as the extinction coefficient, the concentration of LPO was determined as follows: LPO in nmol MDA/g tissue = OD at 532 nm × 320.5128.

Glutathione peroxidase

GSH-Px activity in the liver homogenate was estimated by using DTNB on a supernatant of 10% tissue homogenates [12]. The calculation is as follows: total volume of reaction mixture/volume of sample × OD of test/EC × µmol of GSH/mg. where the extermination coefficient, or EC, was taken to be 13100/M/cm.

Superoxide dismutase assay

SOD activity in the liver homogenate was estimated by the method of Marklund and Marklund (1974) modified by Menami and Yoshikawa (1979) [13, 14]. After appropriately diluting the tissue homogenates, the outcome was multiplied by the dilution factor. The SOD value was represented as a unit per milligram of protein.

Catalase assay

Catalase (CAT) activity in 10% liver tissue homogenate was estimated. In short, tissue samples were diluted 20 times. Using the procedure described, the catalase activity in a 10% liver tissue homogenate was calculated [15]. Tissue samples were diluted twenty times. At 240 nm, the phosphate-H2O2 absorbance was modified to provide an OD value between 0.6 and 0.7. A reference cuvette containing 50?l of phosphate buffer saline (PBS) and 2.95 ml of phosphate buffer - H2O2 was used to measure absorbance at 240 nm after a 50 microliter diluted sample was combined with the solution. Catalase activity levels were expressed in units/mg of protein, and the initial OD

was collected at 30 seconds, with a notation made at that time for a 0.05 (50 unit) drop in initial absorbance.

Gross pathology and histopathology of liver

The liver of all the rats was examined at the end of the experiment for any gross pathological changes. After the trial, the livers of each rat were inspected for any noticeable pathological alterations. Rat liver tissues were preserved for histological analysis using 10% neutral buffer formalin. A histopathological score system (HPS) was created to grade the lesions at several liver locations [16]. With a small modification to the technique, scoring was applied to both the May-Grünwald Giemsa staining procedure and gross pathology. The liver sections were looked for pathological changes and graded on a scale of 0-3 (0= No change, 1 = mild change, 2 = moderate change, 3 = severe change). The maximum score was taken as the most severe inflammatory changes.

May-Grünwald Giemsa staining of liver tissue homogenate

The liver tissue homogenate was stained with May-Grünwald Giemsa according to the protocol described. Under a light microscope, each sample had a minimum of 100 hepatocyte cells, which were counted and examined for cellular abnormalities and alterations. The percentage of normal hepatocytes, the percentage of hepatocytes with a clear or hazy cell wall boundary, the percentage of hepatocytes with aberrant cytoplasmic vacuolation, and the percentage of hepatocytes with pyknotic or necrosed nuclei were the parameters used to evaluate hepatocytes [17].

Statistical analysis

Data was analyzed using two-way ANOVA methods using JMP software. Results are significant at $p<0.05$.

RESULTS AND DISCUSSION

Hematological parameters

The mean ± SE values of hematological parameters have been shown in Tables 2, 3, and 4. The study showed improvement in hematological parameters viz. Hb, PCV, TEC values of Group VI (alginate-coated catechin nanoparticles treated) in comparison to Group II (Disease control). The elevated hematological parameters in treated groups could be attributed to its effect on the bone marrow [18]. Group II showed a significant ($p<0.05$) decrease in the hematological parameters, which may have been caused by toxins directly affecting the hematopoietic system [19] and reducing the impact of carbon tetrachloride on erythropoiesis [20]. The liver also plays a role in erythropoiesis and its dysfunction leads to anemia [21]. In a previous study, the hepatoprotective effect of alginate polymer-coated catechin nanoparticles in a rat model was already evaluated [8]. However, in this study emphasis has been given on the effect of catechin nanoparticles on different doses and dose intervals in rat liver induced with hepatotoxic carbon tetrachloride. Similar improvement in hematological parameters in the dose reduction phase of the trial with a dose rate of 20, 10, and 5 mg/Kg body weight and increased

Table 1. Experimental design for the therapeutic trial on rats (Duration: 4 weeks).

Group (n=6)	Therapeutic group	Treatment
I	Healthy Control	Normal feeding
II	Disease Control	Normal feed + Carbon tetrachloride epigastric lavage with olive oil (1:1 dilution) @ 4ml/kg body weight twice a week [9]
III	Treatment Control-I	Normal feed + Carbon tetrachloride + from day 15th onwards Catechin Analar grade @ 20mg/kg body weight daily [10]
IV	Treatment Control-II	Normal feed + Carbon tetrachloride + from day 15th onwards Blank Alginate analar grade @ 20 mg/kg b.wt daily
V	Conventional Treatment	Normal feed + Carbon tetrachloride + from day 15th onwards Silymarin @ 20 mg/kg daily
VI	Therapy-I	Normal feed + Carbon tetrachloride + from day 15th onwards alginate coated catechin nanoparticles @ 20mg/kg body weight daily
VII	Therapy-II	Normal feed + Carbon tetrachloride +from day 15th onwards alginate coated catechin nanoparticles @ 20mg/kg body weight alternate day
VIII	Therapy-III	Normal feed + Carbon tetrachloride + from day 15th onwards alginate coated catechin nanoparticles @ 20mg/kg body weight every 4th day
IX	Therapy-IV	Normal feed + Carbon tetrachloride + from day 15th onwards alginate coated catechin nanoparticles @ 10mg/kg body weight daily
X	Therapy-V	Normal feed + Carbon tetrachloride + from day 15th onwards alginate coated catechin nanoparticles @ 5mg/kg body weight daily

Table 2. Haematological profile in hepatotoxic rats receiving different treatment (Mean±SE).

Parameter	Group	0 Day	14th Day	28th Day
Haemoglobin (g/dl)	I	14.08 ± 0.24^{aD}	14.00 ± 0.22^{aD}	13.58 ± 0.35^{aCD}
	II	12.83 ± 0.33^{cBCD}	11.33 ± 0.44^{bAB}	9.83 ± 0.31^{aA}
	III	11.60 ± 0.15^{aAB}	11.50 ± 0.43^{aABC}	12.25 ± 0.38^{aBC}
	IV	11.17 ± 0.21^{cA}	10.58 ± 0.24^{abA}	10.42 ± 0.15^{aA}
	V	12.42 ± 0.24^{abABC}	12.50 ± 0.37^{bBCD}	11.30 ± 0.30^{aAB}
	VI	12.88 ± 0.12^{aBCD}	13.48 ± 0.43^{abD}	14.08 ± 0.31^{cD}
PCV (%)	I	42.25 ± 0.72^{aD}	42.12 ± 0.91^{aC}	40.87 ± 1.06^{aCD}
	II	38.50 ± 1.00^{cBCD}	34.00 ± 1.32^{bAB}	29.50 ± 0.92^{aA}
	III	34.80 ± 0.46^{aAB}	34.50 ± 1.28^{aAB}	36.75 ± 1.15^{aBC}
	IV	33.50 ± 0.63^{cA}	31.75 ± 0.72^{abA}	31.25 ± 0.46^{aA}
	V	37.25 ± 0.72^{abABC}	37.50 ± 1.10^{bABC}	33.90 ± 0.91^{aAB}
	VI	38.65 ± 0.37^{aBCD}	40.45 ± 1.29^{abC}	42.25 ± 0.94^{cD}
TEC (10^6/µl)	I	4.60 ± 0.07^{abDE}	4.63 ± 0.05^{bCD}	4.43 ± 0.03^{aB}
	II	3.70 ± 0.05^{bAB}	3.58 ± 0.05^{bA}	2.92 ± 0.19^{aA}
	III	3.87 ± 0.04^{aB}	3.92 ± 0.03^{aB}	4.10 ± 0.35^{aB}
	IV	3.52 ± 0.05^{aA}	3.80 ± 0.04^{bB}	4.50 ± 0.04^{cBC}
	V	4.10 ± 0.04^{aC}	4.60 ± 0.04^{bC}	4.20 ± 0.04^{aB}
	VI	4.40 ± 0.04^{aD}	4.80 ± 0.04^{bD}	6.10 ± 0.04^{eC}
TLC (10^3/µl)	I	13.42 ± 3.00^{aA}	9.54 ± 1.29^{aA}	9.30 ± 1.12^{aA}
	II	11.47 ± 1.49^{aA}	12.39 ± 1.55^{aBA}	12.64 ± 1.21^{aA}
	III	19.23 ± 5.08^{bA}	13.76 ± 3.16^{abAB}	9.22 ± 2.64^{aA}
	IV	14.95 ± 3.69^{abA}	20.29 ± 2.32^{bB}	10.82 ± 2.35^{aA}
	V	17.79 ± 2.26^{aA}	11.77 ± 2.70^{aAB}	8.42 ± 1.57^{aA}
	VI	15.17 ± 4.29^{aA}	15.44 ± 2.23^{abA}	8.91 ± 1.79^{aA}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

Table 3. Haematological profile in hepatotoxic rats receiving reduced dose of alginate coated catechin nanoparticles (Mean±SE).

Parameter	Group	0 Day	14th Day	28th Day
Haemoglobin(g/dl)	VI	14.08 ± 0.24^{aD}	14.00 ± 0.22^{aD}	13.58 ± 0.35^{aCD}
	VII	13.00 ± 0.37^{aBCD}	13.25 ± 0.39^{aBCD}	13.58 ± 0.32^{aCD}
	VIII	12.90 ± 0.38^{aBCD}	13.02 ± 0.38^{aBCD}	13.50 ± 0.30^{aCD}
PCV (%)	VI	38.65 ± 0.37^{aBCD}	40.45 ± 1.29^{abC}	42.25 ± 0.94^{cD}
	VII	39.00 ± 1.10^{aBCD}	39.75 ± 1.16^{aBC}	40.75 ± 0.97^{aCD}
	VIII	38.70 ± 1.15^{aBCD}	39.05 ± 1.13^{aBC}	40.50 ± 0.90^{aCD}
TEC (10^6/µl)	VI	4.40 ± 0.04^{aD}	4.80 ± 0.04^{bD}	6.10 ± 0.04^{eC}
	VII	4.77 ± 0.05^{aE}	5.10 ± 0.04^{bE}	5.77 ± 0.04^{cdB}
	VIII	4.72 ± 0.03^{aEA}	5.00 ± 0.04^{bE}	5.12 ± 0.14^{cdB}
TLC (10^3/µl)	VI	15.17 ± 4.29^{aA}	15.44 ± 2.23^{abA}	8.91 ± 1.78^{aA}
	VII	15.43 ± 2.19^{aA}	13.43 ± 3.16^{aA}	7.08 ± 1.49^{bA}
	VIII	12.26 ± 3.00^{abA}	15.69 ± 3.29^{aA}	5.99 ± 1.23^{bA}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

Table 4. Haematological profile in hepatotoxic rats with increased dose interval of alginate coated catechin nanoparticles (Mean±SE).

Parameter	Group	0 Day	14th Day	28th Day
Haemoglobin(g/dl)	VI	4.40±0.04^{aD}	4.80±0.04^{bD}	6.10±0.04^{eC}
	IX	4.40±0.04^{aD}	5.13±0.04^{bE}	5.17±0.05^{dB}
	X	4.13±0.03^{aC}	4.58±0.03^{bC}	5.10±0.04^{cdC}
PCV (%)	VI	38.65±0.37^{aBCD}	40.45±1.29^{abC}	42.25±0.94^{cD}
	IX	38.50±1.43^{aBCD}	39.35±2.20^{aBC}	40.05±1.74^{aCD}
	X	38.70±0.69^{aBCD}	39.65±0.57^{aBC}	39.90±0.64^{aCD}
TEC (10^6/µl)	VI	4.40±0.04^{aD}	4.80±0.04^{bD}	6.10±0.04^{eC}
	IX	4.54±0.30^{aD}	5.30±0.51^{abA}	6.00±0.04^{eC}
	X	4.29±0.04^{aD}	5.13±0.04^{bE}	5.17±0.05^{dB}
TLC (10^3/µl)	VI	15.17±4.29^{aA}	15.44±2.23^{abA}	8.91±1.78^{bA}
	IX	13.53±4.30^{aA}	13.43±3.16^{aA}	7.99±0.83^{bA}
	X	11.82±2.18^{aA}	13.29±3.98^{aA}	7.77±1.11^{bA}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 5. Serum profile in hepatotoxic rats receiving different treatments (Mean±SE).

Parameter	Group	0 Day	14th Day	28th Day
SGPT(U/L)	I	75.85±5.07^{aAB}	74.29±8.01^{aAB}	73.22±6.50^{aAB}
	II	86.22±9.48^{aAB}	101.82±8.22^{aBCD}	106.08±7.46^{aB}
	III	96.53±4.17^{aB}	99.76±4.08^{aB}	110.84±7.12^{aD}
	IV	82.43±8.45^{aAB}	94.12±7.89^{aAB}	96.16±1.85^{aBC}
	V	57.40±7.20^{aAB}	67.85±7.43^{aA}	68.78±8.21^{aAB}
	VI	83.72±7.80^{bAB}	85.59±7.40^{bAB}	47.57±3.56^{aA}
SGOT(U/L)	I	52.91±3.93^{aA}	55.81±5.98^{aA}	56.71±4.88^{aA}
	II	86.07±3.22^{aBC}	89.20±3.50^{aB}	94.60±2.70^{aBC}
	III	93.21±3.51^{aC}	100.96±3.07^{abB}	110.14±4.79^{bC}
	IV	90.55±10.89^{aBC}	99.12±10.80^{aB}	95.49±9.98^{aBC}
	V	84.55±4.19^{aBC}	87.10±3.80^{aB}	87.71±4.13^{aBC}
	VI	84.08±8.11^{abBC}	95.72±7.71^{bB}	59.69±5.76^{aA}
ALP(U/L)	I	92.53±1.32^{aA}	87.76±2.78^{aA}	87.54±2.42^{aA}
	II	150.39±1.94^{aCDE}	180.60±2.46^{bE}	194.80±2.55^{cE}
	III	146.38±1.64^{aCDE}	152.19±2.89^{aCD}	179.66±3.80^{bDE}
	IV	128.37±0.67^{aB}	132.13±1.64^{aBC}	146.61±4.25^{bBC}
	V	160.91±3.75^{aE}	161.63±3.69^{aDE}	185.90±3.51^{bE}
	VI	138.95±1.06^{bBCD}	128.17±7.66^{abB}	117.22±3.15^{aAB}
Total Protein(g/dl)	I	6.36±0.05^{bAB}	6.11±0.00^{aAB}	6.35±.02^{bAB}
	II	5.89±.03^{aAB}	5.38±.02^{bA}	4.70±.03^{aA}
	III	5.85±.03^{aAB}	5.85±.03^{aAB}	6.09±.33^{aAB}
	IV	5.52±.37^{aA}	5.99±.19^{aAB}	7.35±.42^{bB}
	V	7.02±.62^{aAB}	7.60±.66^{aB}	7.27±.58^{aB}
	VI	7.34±.52^{aB}	6.68±.58^{aAB}	7.61±.48^{aB}
Albumin(g/dl)	I	3.55±0.32^{aA}	3.76±0.44^{aB}	3.89±0.37^{aB}
	II	2.98±0.39^{aA}	2.27±0.16^{aB}	2.12±0.03^{aA}
	III	3.02±0.17^{aA}	3.05±0.19^{aB}	2.84±0.24^{aAB}
	IV	3.52±0.28^{aA}	3.51±0.16^{aB}	3.63±0.88^{aAB}
	V	3.17±0.12^{bA}	1.77±0.22^{aA}	3.42±0.39^{aAB}
	VI	2.92±0.35^{aA}	2.79±0.46^{aA}	3.71±0.38^{aA}
Total Bilirubin (mg/dl)	I	0.18±0.05^{aA}	0.20±0.04^{aA}	0.16±0.03 ^{aA}
	II	0.21±0.04^{aA}	0.34±0.02^{bA}	0.41±0.03^{bB}
	III	0.20±0.06^{aA}	0.24±0.08^{aA}	0.24±0.08^{aAB}
	IV	0.18±0.06^{aA}	0.19±0.07^{aA}	0.19±0.06^{aAB}
	V	0.35±0.06 ^{aA}	0.38±0.05^{aA}	0.39±0.05^{aAB}
	VI	0.17±0.06^{aA}	0.28±0.07^{aA}	0.20±0.05^{aAB}
Direct Bilirubin (mg/dl)	I	0.12±0.04^{aA}	0.04±0.01^{aA}	0.05±0.02^{aA}
	II	0.17±0.01^{aAB}	0.26±0.01^{bAB}	0.26±0.01^{bAB}
	III	0.21±0.02^{aAB}	0.21±0.14^{aA}	0.19±0.13^{aA}
	IV	0.17±0.01^{aAB}	0.18±0.04^{aAB}	0.17±0.03^{aA}
	V	.36±0.05^{aB}	0.21±0.03^{aA}	0.21±0.04^{aA}
	VI	0.15±0.03^{aAB}	0.24±0.04^{aAB}	0.17±0.03^{aA}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 6. Serum biochemical profile in hepatotoxic rats receiving reduced dose of alginate coated catechin nanoparticles (Mean±SE)

Parameter	Group	0 Day	14th Day	28th Day
SGPT(U/L)	VI	83.72 ± 7.80^{bAB}	85.59 ± 7.40^{bAB}	47.57 ± 3.56^{aA}
	VII	71.03 ± 1.64^{abAB}	79.89 ± 5.45^{bAB}	64.07 ± 4.22^{aA}
	VIII	67.51 ± 1.81^{aAB}	76.41 ± 2.80^{aAB}	70.19 ± 3.56^{aAB}
SGOT(U/L)	VI	84.08 ± 8.11^{abBC}	95.72 ± 7.71^{bB}	59.69 ± 5.76^{aA}
	VII	77.64 ± 4.54^{aABC}	80.12 ± 5.86^{aAB}	68.91 ± 6.09^{aAB}
	VIII	72.82 ± 7.11^{aABC}	85.94 ± 2.78^{aB}	72.43 ± 7.32^{aAB}
Total Protein (g/dl)	VI	$7.34\pm.52^{aB}$	$6.68\pm.58^{aAB}$	$7.61\pm.48^{aB}$
	VII	$6.58\pm.33^{aAB}$	$5.89\pm.47^{aAB}$	$6.00\pm.40^{aAB}$
	VIII	$6.17\pm.87^{aAB}$	5.84 ± 1.70^{aAB}	$6.04\pm.91^{aAB}$
Albumin (g/dl)	VI	2.92 ± 0.35^{aA}	2.79 ± 0.46^{aA}	3.71 ± 0.38^{aA}
	VII	3.16 ± 0.12^{aA}	2.25 ± 0.47^{aA}	3.27 ± 0.26^{aA}
	VIII	3.55 ± 0.21^{bA}	2.15 ± 0.53^{aA}	3.44 ± 0.39^{bA}
Total Bilirubin (mg/dl)	VI	0.18 ± 0.05^{aA}	0.20 ± 0.04^{aA}	0.16 ± 0.03^{aA}
	VII	0.30 ± 0.03^{aA}	0.33 ± 0.07^{aA}	0.24 ± 0.05^{aAB}
	VIII	0.21 ± 0.03^{aA}	0.23 ± 0.06^{aA}	0.23 ± 0.06^{aAB}
Direct Bilirubin (mg/dl)	VI	0.15 ± 0.03^{aAB}	0.24 ± 0.04^{aAB}	0.17 ± 0.03^{aA}
	VII	0.19 ± 0.04^{aAB}	0.21 ± 0.06^{aAB}	0.19 ± 0.02^{aA}
	VIII	0.19 ± 0.06^{aAB}	0.21 ± 0.14^{aA}	$0.20\pm.04^{aAB}$

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

Table 7. Serum biochemical profile in hepatotoxic rats with increased duration of dose interval of alginate coated catechin nanoparticles (Mean±SE).

Parameter	Group	0 Day	14th Day	28th Day
SGPT (U/L)	VI	83.72 ± 7.80^{bAB}	85.59 ± 7.40^{bAB}	47.57 ± 3.56^{aA}
	IX	69.96 ± 5.22^{aAB}	76.73 ± 4.42^{aAB}	66.72 ± 2.97^{aAB}
	X	74.82 ± 9.44^{aAB}	92.89 ± 3.88^{aAB}	80.22 ± 3.31^{aABC}
SGOT (U/L)	VI	84.08 ± 8.11^{abBC}	95.72 ± 7.71^{bB}	59.69 ± 5.76^{aA}
	IX	64.12 ± 5.93^{aAB}	74.36 ± 5.71^{aAB}	69.52 ± 5.06^{aAB}
	X	79.82 ± 3.36^{aABC}	85.40 ± 4.09^{aB}	72.89 ± 4.34^{aAB}
Total Protein (g/dl)	VI	$7.34\pm.52^{aB}$	$6.68\pm.58^{aAB}$	$7.61\pm.48^{aB}$
	IX	$6.37\pm.57^{bAB}$	$5.32\pm.26^{aA}$	$6.19\pm.45^{aAB}$
	X	$6.08\pm.21^{aAB}$	$4.83\pm.88^{aA}$	$5.91\pm.38^{bAB}$
Albumin (g/dl)	VI	2.92 ± 0.35^{aA}	2.79 ± 0.46^{aA}	3.71 ± 0.38^{aA}
	IX	3.45 ± 0.27^{bA}	2.27 ± 0.49^{aA}	3.24 ± 0.57^{bA}
	X	3.43 ± 0.21^{bA}	1.80 ± 0.53^{aA}	3.43 ± 0.38^{bA}
Total Bilirubin (mg/dl)	VI	0.18 ± 0.05^{aA}	0.20 ± 0.04^{aA}	0.16 ± 0.03^{aA}
	IX	0.21 ± 0.06^{aA}	0.24 ± 0.02^{aA}	0.24 ± 0.02^{aAB}
	X	0.18 ± 0.05^{aA}	0.27 ± 0.05^{aA}	0.32 ± 0.06^{aAB}
Direct Bilirubin (mg/dl)	VI	0.15 ± 0.03^{aAB}	0.24 ± 0.04^{aAB}	0.17 ± 0.03^{aA}
	IX	0.23 ± 0.08^{aAB}	0.26 ± 0.08^{aAB}	0.21 ± 0.05^{aAB}
	X	0.20 ± 0.06^{aAB}	0.27 ± 0.09^{aAB}	0.26 ± 0.08^{aAB}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

Table 8. Oxidative stress indices in hepatotoxic rats receiving different treatment (Mean±SE).

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
LPO (nMMda/ml)	45.02±0.42^{a}	90.08±0.13^{c}	53.61±7.30^{ab}	47.38±0.23^{ab}	55.05±0.15^{ab}	56.18±0.14^{b}
SOD (unit/mg protein)	14.25±0.4^{f}	1.78±0.06^{a}	2.35±0.05^{a}	2.03±0.02^{a}	7.05±0.01^{cd}	9.43±0.08^{e}
GSH (mM/mg protein)	0.72±0.01^{e}	0.24±0.01^{a}	0.35±0.01^{b}	0.38±0.02^{b}	0.85±0.00^{e}	0.75±0.03^{e}
Catalase (unit/mg protein)	2.34±0.04^{e}	1.25±0.00^{a}	3.37±0.01^{f}	1.72±0.01^{c}	1.22±0.00^{a}	2.12±0.00^{d}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 9. Oxidative stress indices in hepatotoxic rats received reducing dose of Alginate coated catechin nanoparticles (Mean±SE).

Parameter	Group VI	Group IX	Group X
LPO (nMMda/ml)	56.18±0.14^{b}	53.81±0.22^{ab}	85.28±0.29^{c}
SOD (unit/mg protein)	9.43±0.08^{e}	2.20±0.05^{a}	4.92±0.57^{b}
GSH (mM/mg protein)	0.75±0.03^{b}	0.56±0.01^{c}	0.65±0.01^{d}
Catalase (unit/mg protein)	2.12±0.00^{d}	1.25±0.01^{a}	1.66±0.01^{c}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 10. Oxidative stress indices in hepatotoxic rats with increase duration of dose interval of alginate coated catechin nanoparticles (Mean±SE).

Parameter	Group VI	Group VII	Group VIII
LPO (nMMDA/ml)	56.18±0.14^{b}	94.40±0.30^{c}	85.45±0.09^{c}
SOD (unit/mg protein)	9.43±0.08^{e}	6.19±0.03^{c}	7.24±0.06^{d}
GSH (mM/mg protein)	0.75±0.03^{e}	0.71±0.02^{de}	0.71±0.02^{d}
Catalase (unit/mg protein)	2.12±0.00^{d}	1.72±0.01^{c}	1.33±0.01^{b}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 11. Percentage hepatocyte population by May-Grünwald Giemsa staining in rats receiving different treatment (Mean±SE).

Parameter (%)	Group I	Group II	Group III	Group IV	Group V	Group VI
Normal hepatocyte cell	68.41±2.99^{h}	3.63±0.27^{a}	11.59±0.33^{b}	27.43±1.46^{de}	34.35±1.02^{f}	41.92±0.95^{g}
Distinct hepatocyte cell wall boundary	66.83±2.51^{e}	28.50±1.07^{b}	16.30±0.44^{a}	47.94±0.72^{d}	52.69±2.04^{c}	41.54±0.45^{c}
Indistinct hepatocyte cell wall boundary	22.94±2.19^{a}	75.57±1.33^{f}	56.73±1.31^{e}	45.52±0.91^{bc}	53.76±1.55^{ed}	43.41±0.83^{b}
Abnormal vacuolation	7.72±0.72^{a}	46.57±1.07^{d}	22.03±1.88^{b}	35.36±1.30^{c}	21.47±0.65^{b}	24.47±0.57^{b}
Pyknotic hepatocyte cell	3.86±0.31^{a}	24.80±0.53^{f}	21.13±0.42^{e}	7.61±0.59^{cd}	5.89±0.25^{bc}	7.67±0.38^{cd}
Necrosed hepatocyte nucleus	2.03±0.27^{a}	27.99±0.38^{f}	11.77±0.34^{e}	5.59±0.33^{c}	5.61±0.22^{c}	4.68±0.25^{cb}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at p<0.05.

Table 12. Percentage hepatocyte population by May-Grünwald Giemsa staining in hepatotoxic rats received reducing dose of CH-NP (Mean±SE).

Parameter (%)	Group VI	Group IX	Group X
Normal hepatocyte cell	41.92±0.95^{g}	17.50±0.36^{c}	25.85±0.33^{d}
Distinct cell wall boundary	41.54±0.45^{c}	50.68±0.70^{d}	41.29±0.47^{c}
Indistinct cell wall boundary	43.41±0.83^{b}	43.64±0.61^{b}	53.48±1.87^{ed}
Abnormal vacuolation in cytoplasm	24.47±0.57^{b}	24.85±0.79^{b}	41.95±1.11^{d}
Pyknotic hepatocyte nucleus	7.67±0.38^{cd}	4.12±0.23^{ab}	8.70±0.30^{d}
Necrosed hepatocyte nucleus	4.68±0.25^{cb}	4.00±0.23^{b}	7.41±0.49^{d}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

Table 13. Percentage hepatocyte population by May-Grunwald Giemsa staining in hepatotoxic rats with increase duration of dose interval of CH-NP (Mean±SE).

Parameter (%)	Group VI	Group VII	Group VIII
Normal hepatocyte cell	41.92±0.95^{g}	31.95±0.75^{ef}	27.76±0.36^{de}
Distinct cell wall boundary	41.54±0.45^{c}	42.41±0.56^{c}	49.92±0.46^{d}
Indistinct cell wall boundary	43.41±0.83^{b}	49.97±0.46^{cd}	41.65±0.56^{b}
Abnormal vacuolation in cytoplasm	24.47±0.57^{b}	43.45±0.48^{d}	24.72±0.44^{b}
Pyknotic hepatocyte nucleus	7.67±0.38^{cd}	4.05±0.23^{a}	5.55±0.39^{ab}
Necrosed hepatocyte nucleus	4.68±0.25^{cb}	7.41±0.27^{d}	4.70±0.41^{cb}

Mean ± SE values for a given parameter in the same row (small letter) and column (capital letter) with the same superscript do not differ at $p<0.05$.

dosing interval phase of the trial with 20mg/Kg body weight on daily alternate days and every 4th day implicate nano-formulation of catechin could exert an identical protective effect on bone marrow [4].

Serum biochemical profile

The mean ± SE values of serum biochemical parameters have been presented in Tables 5, 6, and 7. A significant ($p<0.05$) decrease in the serum enzyme activity of SGOT, SGPT, and ALP was noticed in Group VI as compared to Group II (diseased group). These results were in agreement with Hung *et al.* (2006) [22] who stated that carbon tetrachloride-treated rats cased liver damage which was indicated by a significant increase in SGOT, SGPT, and ALP levels in serum as compared to the control group. Total protein, albumin, total bilirubin, and direct bilirubin values of Group II were also negatively altered and the same parameters showed significant ($p<0.05$) improvement in Group VI treated with alginate-coated catechin nanoparticles. This confirmed previous report of improvement in the alginate-coated catechin-treated group indicating the stabilization of the plasma membrane and repair of hepatic tissue by the catechin nanoparticle [8].

In the dose reduction trial, the serum enzyme activity of SGOT, SGPT, and ALP showed similar values depicting low dose rates of catechin nanostructure that were able to stabilize and repair hepatic tissue damage caused by carbon tetrachloride. A similar improvement in values of total protein, albumin, total bilirubin, and direct bilirubin was noted in the dose reduction phase which explained the ability of nanodrug to counteract the toxicity caused by carbon tetrachloride even in a reduced dose rate. Increasing the dosing interval of catechin nanoparticles depicted a similar capability to stabilize and repair hepatic tissue damage which was indicated by a reduction in the value of SGPT, SGOT, and ALP [23].

Assays of oxidative stress markers in liver tissue sample

Mean±SE values of the oxidative stress markers in the liver tissue samples are presented in Tables 8, 9, and 10. The present study revealed elevated levels of lipoxidation (LPO) in the livers of all rats administered carbon tetrachloride, suggesting that lipid peroxidation is the underlying cause of liver damage. Among the treatment groups, both Group III and Group VI showed

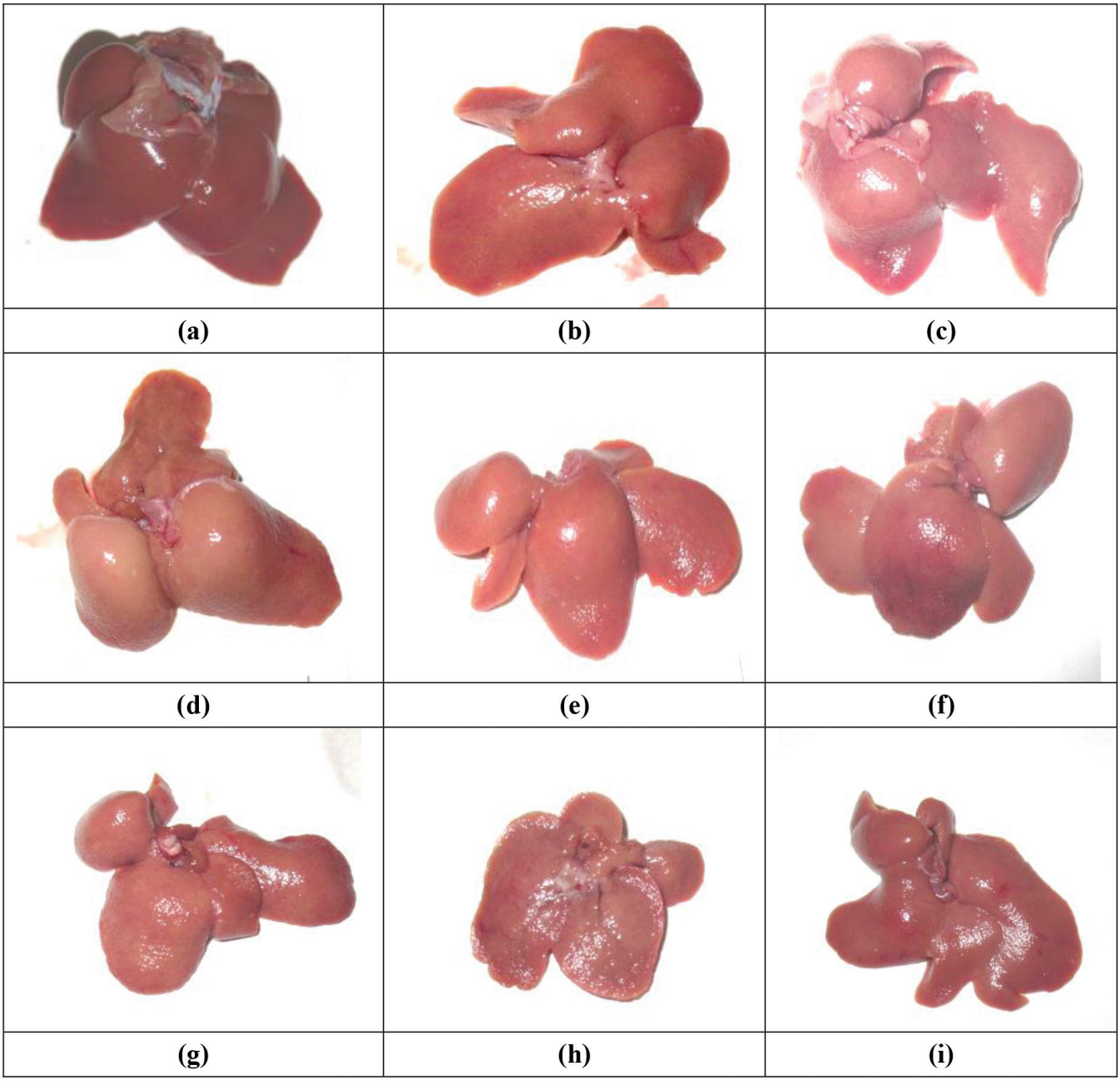

Fig. 1. Gross pathology of liver in different groups.

[(From left to right a-i) 1a: Group I-Normal architecture liver; 1b: Group II-Cirrhosis in liver; 1c: GroupIII-Mild hepatomegaly; 1d: GroupIV- Moderate hepatomegaly; 1e: GroupV- Moderate hepatomegaly; 1f: GroupVI-Apparently normal architecture; 1g-1i: GroupVII-X Apparently normal architecture with mild hepatomegaly].

a significant ($p<0.05$) decrease in the level of lipid peroxidation when compared with Group II indicating better antioxidant activity of catechin as annular grade and polymer nanoform. Comporti (1985) stated that GSH was helpful for the removal of free radicals such as hydrogen peroxide and superoxide radicals, alkoxy radicals, detoxification of foreign chemicals, and biotransformation of drugs [24].

Rats of Group V and Group VI showed a significantly increased level of SOD denoting a better effect of Alginate coated catechin nanoparticles and standard. Similar findings were also reported [8, 25]. Endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) have shown similar improvement in the dose reduction phase of the trial implicating the ability of

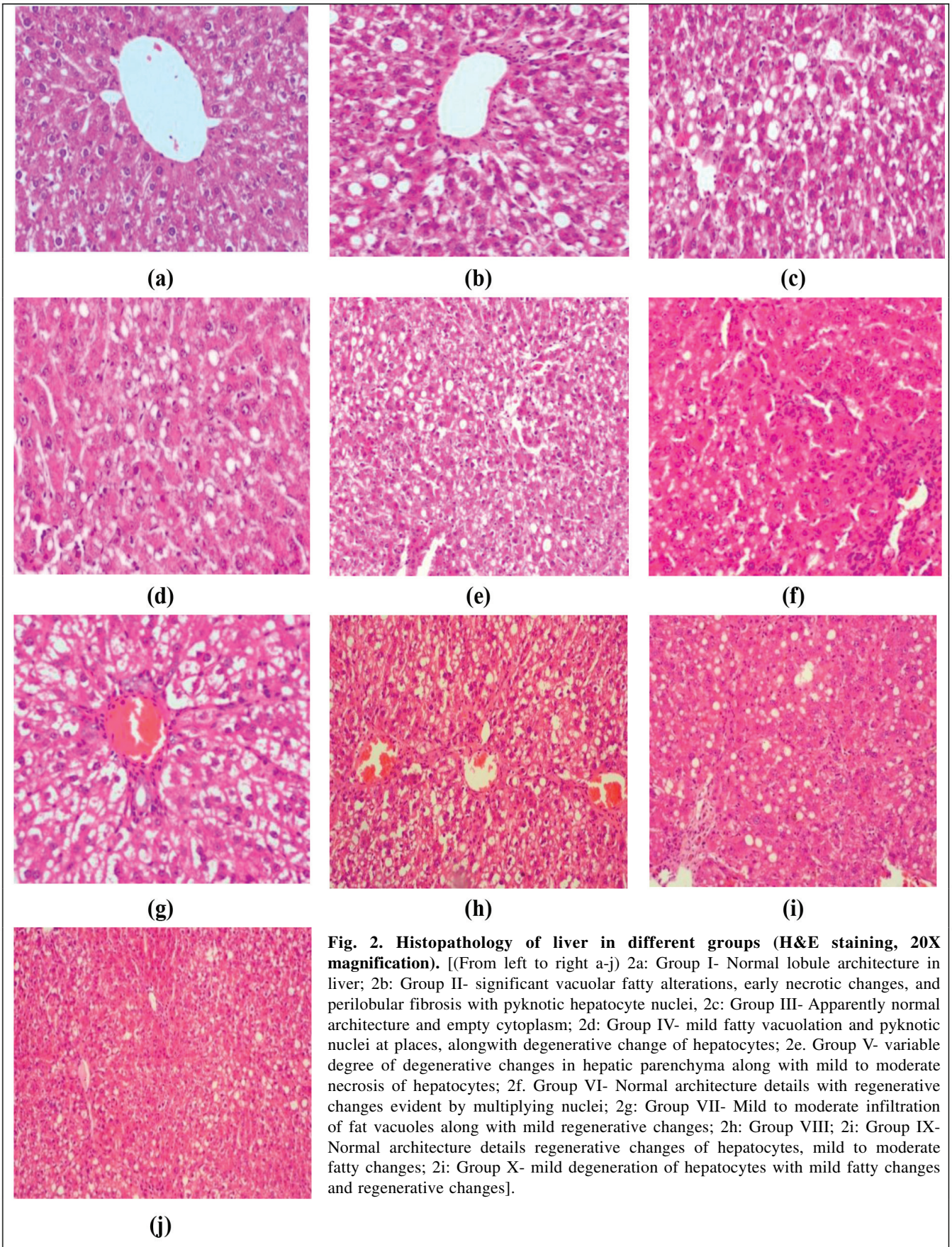

Fig. 2. Histopathology of liver in different groups (H&E staining, 20X magnification). [(From left to right a-j) 2a: Group I- Normal lobule architecture in liver; 2b: Group II- significant vacuolar fatty alterations, early necrotic changes, and perilobular fibrosis with pyknotic hepatocyte nuclei, 2c: Group III- Apparently normal architecture and empty cytoplasm; 2d: Group IV- mild fatty vacuolation and pyknotic nuclei at places, alongwith degenerative change of hepatocytes; 2e. Group V- variable degree of degenerative changes in hepatic parenchyma along with mild to moderate necrosis of hepatocytes; 2f. Group VI- Normal architecture details with regenerative changes evident by multiplying nuclei; 2g: Group VII- Mild to moderate infiltration of fat vacuoles along with mild regenerative changes; 2h: Group VIII; 2i: Group IX- Normal architecture details regenerative changes of hepatocytes, mild to moderate fatty changes; 2i: Group X- mild degeneration of hepatocytes with mild fatty changes and regenerative changes].

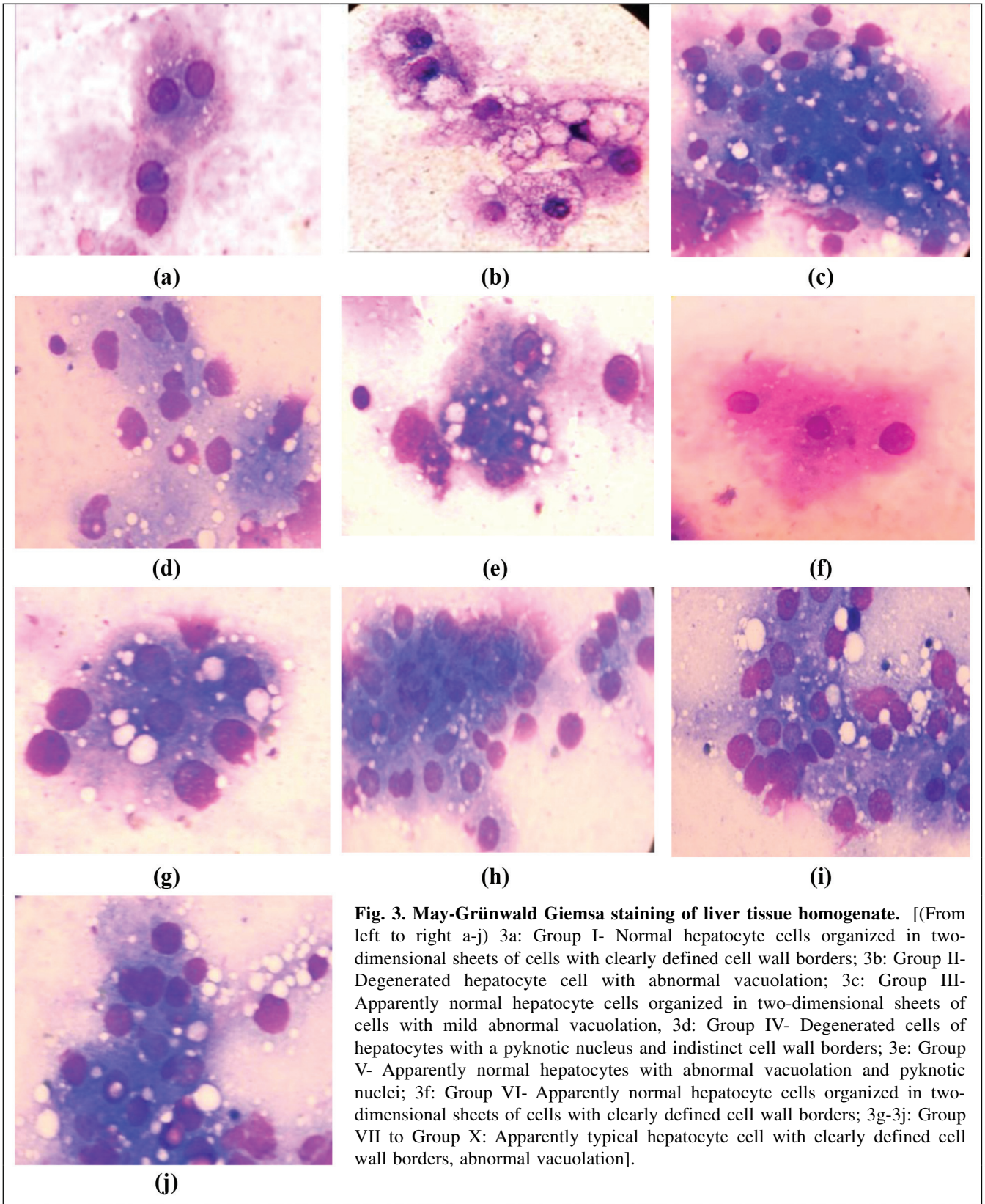

Fig. 3. May-Grünwald Giemsa staining of liver tissue homogenate. [(From left to right a-j) 3a: Group I- Normal hepatocyte cells organized in two-dimensional sheets of cells with clearly defined cell wall borders; 3b: Group II- Degenerated hepatocyte cell with abnormal vacuolation; 3c: Group III- Apparently normal hepatocyte cells organized in two-dimensional sheets of cells with mild abnormal vacuolation, 3d: Group IV- Degenerated cells of hepatocytes with a pyknotic nucleus and indistinct cell wall borders; 3e: Group V- Apparently normal hepatocytes with abnormal vacuolation and pyknotic nuclei; 3f: Group VI- Apparently normal hepatocyte cells organized in two-dimensional sheets of cells with clearly defined cell wall borders; 3g-3j: Group VII to Group X: Apparently typical hepatocyte cell with clearly defined cell wall borders, abnormal vacuolation].

alginate coated catechin nanoparticles to enhance the antioxidant capacity of the liver equally at 20, 10 and 5 mg/Kg body weight. However, dose reduction has shown less effect to counteract an increase in LPO value due to carbon tetrachloride4-induced hepatic toxicity.

In summary, compared to Group III, which received annular grade catechin hydrate, rats in Group VI treated with alginate-coated catechin nanoparticles showed greater liver tissue antioxidant activity. Spizzirri *et al.* (2010) discovered that adding catechin to alginate enhanced its antioxidant properties [26].

It was concluded that the rats treated with sodium alginate-coated catechin nanoparticles revealed better liver tissue antioxidant activity [8]. Rats of Group VI also showed better antioxidant activity than the standard group (Group V) which revealed increased oral bioavailability [27, 28] and enhanced cellular uptake on the conversion of catechin hydrate to nano-size polymer [29, 30]. The study revealed that alginate-coated catechin nanoparticles at dose rates of 20, 10, and 5mg/Kg body weight have similar remarkable potential to ameliorate carbon tetrachloride 4-induced hepatotoxicity. The study also revealed the ability of alginate-coated catechin nanoparticles to counteract the hepatotoxicity caused by carbon tetrachloride on alternate-day dosing like daily dosing.

Gross pathology of the liver

Upon gross examination, Group II liver revealed mild cirrhosis and hepatomegaly, along with a few white areas that were likely caused by fat deposition. Mice treated acutely with carbon tetrachloride showed fatty and necrotic alterations in their liver [31]. Among the treated groups, Group VI showed no apparent abnormality and this could be attributed to increased antioxidant activity and cellular uptake on nanoformulation of catechin hydrate [29, 30].

Histopathological observation

The histopathology of the liver of different groups of rats is shown in Fig. 2. Hepatic damage caused by carbon tetrachloride is characterized by fatty changes with small to large vacuoles mild perilobular fibrosis and severe degeneration [32, 33]. Alginate-coated catechin nanoparticles at a dose of 20 mg/kg body weight were found to have a hepatoprotective effect in an in-vivo study. This effect was demonstrated by the nuclei of the damaged liver multiplying. It was also observed a regenerative effect in the rats receiving sodium alginate-coated catechin nanoparticles [8]. In a dose reduction trial, similar regenerative changes of hepatocytes noted in rats treated with 20, 10, and 5 mg/Kg body weight depicted the ability of nano catechin to exert a protective effect even at a reduced dose rate. In the increasing dose interval study, comparable improvement was noted in rats with everyday dosing (Group VI) and alternate day dosing (Group IX) with a moderate increase in degenerative changes in alternate day dosing which explained the sustained releasing property of catechin from nanoform. But in every 4th day dosing rats (Group X), less improvement was noted as compared to every day dosing. The ameliorative effect of the liver in alginate-coated catechin nanoparticles can be attributed to the antioxidant activity of catechin hydrate [34] and a similar hepatoprotective effect was reported by Sankar *et al.* (2013) who concluded that nano flavonoid aided better regenerative effect [30].

May-Grunwald Giemsa staining of the liver tissue homogenate

Tables 11, 12, 13, and Fig. 3 displayed the Mean±SE results of the May-Grünwald Giemsa staining liver tissue homogenate. Normal hepatocyte cell study by May-Grünwald Giemsa staining in rats depicted normal architecture in rats of Group I followed by GroupVI, Group V, and Group III in decreasing order and poor in Group II. These findings were in agreement with the histopathological changes which depicted improvement in the livers of GroupVI rats than other groups. Hepatocyte cell wall boundary study revealed supremacy in the liver of Group VI than Group III which reveals the better hepatoprotectant efficacy of Alginate coated catechin nanoparticles than plain catechin hydrate. A study of indistinct hepatocyte cell boundary revealed the lowest percentage in the liver of GroupVI as compared to the rat receiving catechin alone, the disease control group, and the standard treated group. A similar trend of hepatoprotective effect with alginate-coated catechin nanoparticles was found with regards to pyknotic hepatocyte nucleus and necrosed hepatocytes. About abnormal vacuolation in the cytoplasm, rats of Group V showed similar results as compared to Group III. It was reported that significantly lower abnormal vacuolation in the hepatocytes cytoplasm of the rats receiving catechin nanoparticles [8].

In the dose reduction trial of alginate-coated catechin nanoparticles, the groups that received 20 mg/Kg body weight (Group VI) exhibited better improvement followed by Group VIII and Group VI in hepatocytes morphology against carbon tetrachloride4 toxicity. A reduced percentage of hepatocyte parameters experiencing injury or death, together with a percentage of hepatocytes with defined cell wall borders and minimal intracellular fat and glycogen, was used to determine if hepatocyte regeneration or improvement

was successful. The study found that alginate-coated catechin nanoparticles were more effective in treating liver disease. This was ascribed to the regulated release and improved hepatic cellular uptake of the nanoformulation of catechin hydrate.

CONCLUSION

In vivo trial of catechin hydrate nanoparticles in a rat model depicted improvement in hematological, biochemical parameters, and oxidative stress markers on the 28th day in the rats treated with catechin nanoparticles as compared to other treated groups. The hepatoprotective effect of alginate-coated catechin nanoparticles on dose reduction trials with a dose rate of 20, 10, and 5 mg/Kg body weight revealed comparable results. The study concluded that nano-formulation of catechin using alginate polymer has shown a greater potential to ameliorate hepatotoxicity at a lower dose rate. Synthesized alginate-coated catechin nanoparticles may be a better option against the conventional therapy with Silymarin to ameliorate hepatopathy.

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