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Design of cissus–alginate microbeads revealing mucoprotection properties in anti-inflammatory therapy

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ABSTRACT

Cissus gum has been employed as polymer with sodium alginate in the formulation of diclofenac microbeads and the *in vivo* mucoprotective properties of the polymer in anti-inflammatory therapy assessed in rats with carrageenan-induced paw edema in comparison to diclofenac powder and commercial diclofenac tablet. A full 2^3 factorial experimental design has been used to investigate the influence of concentration of cissus gum (X_1); concentration of calcium acetate (X_2) and stirring speed (X_3) on properties of the microbeads. Optimized small discrete microbeads with size of 1.22 ± 0.10 mm, entrapment efficiency of 84.6% and t_{80} of 15.2 ± 3.5 h were obtained at ratio of cissus gum:alginate (1:1), low concentration of calcium acetate (5% w/v) and high stirring speed (400 rpm). *In vivo* studies showed that the ranking of percent inhibition of inflammation after 3 h was diclofenac powder > commercial tablet = cissus > alginate. Histological damage score and parietal cell density were lower while crypt depth and mucosal width were significantly higher ($p < 0.05$) in the groups administered with the diclofenac microbeads than those administered with diclofenac powder and commercial tablet, suggesting the mucoprotective property of the gum. Thus, cissus gum could be suitable as polymer in the formulation of non-steroidal anti-inflammatory drugs ensuring sustained release while reducing gastric side effects.

1. Introduction

Natural biodegradable polymers such as starches and gums present a fairly broad area of active research in controlled drug delivery [1,2]. The advantage of these natural polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body *via* normal metabolic pathways [3,4]. These natural polymers are hydrophilic and are more amenable to physical and chemical modifications using simple processes than the widely used synthetic polymers [5–8].

Alginate, a linear hetero-polysaccharide, extracted from different types of algae has been used for the formulation of microbeads under mild process conditions. However, some of the disadvantages attributed to alginate polymers include their susceptibility to degradation in acidic environments of the stomach and the fact that when alginate gel is formed in the presence of calcium ions, the integrity of the beads may be affected by monovalent ions or chelating agents such as phosphates, lactates and citrates, which absorb calcium ions [9]. Furthermore, they form microbeads, which have cracked and porous surfaces that can lead to relatively fast diffusion of moisture and other fluids, thus reducing the barrier properties in unfavorable environmental conditions [10]. Blending alginate with natural polymers such as gums and starches is a recent innovation that has been shown to be effective in overcoming many of these limitations by enhancing encapsulation efficiency and drug release properties of microbeads [4,11–13].

Cissus gum, obtained from the plant *Cissus pulpunea* Guill and Perr, Family Ampelidacae, is one of the numerous underutilized gums distributed in many parts of Africa, especially the savannah region. The plant has great propensity for retaining water and thus remains fresh almost throughout the year. Cissus gum is popularly referred to as food gum with a wide range of local applications in Africa where it is used as soup thickener and remedy for indigestion [14]. Cissus gum is a hydrophilic polysaccharide that swells rapidly

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in cold water and a 2% dispersion has been shown to attain a viscosity of 11.6 Pa s. It has a particle density of 1.59 g cm^3 and glass transition temperature of 264.2 °C [12]. The gum has been evaluated as a binder in pharmaceutical tablets where it was reported to produce tablets with high mechanical strength and slow drug release properties [15]. Cissus gum along with three other natural gums namely khaya, albizia and irvingia gums, has also been characterized and used, for the formulation of microbeads by ionic gelation method using zinc chloride as the chelating agent [12]. However, relatively low entrapment efficiency and short dissolution times were obtained for cissus gum, in comparison to sodium alginate and the other natural gum-alginate blends that were evaluated. Of the four natural gums evaluated, cissus gum showed more promise due to its use as remedy for indigestion suggesting probable mucoprotective properties, which could be useful in anti-inflammatory therapy.

Thus in the present study, optimized diclofenac sodium microbeads have been formulated from blends of cissus gum and sodium alginate using a total polymer concentration of 2.50% w/v, polymer to drug ratio of 4:1, and calcium acetate as cross-linking agent. The optimized microbeads were administered orally to rats with carrageenan-induced inflammation exhibited as paw edema and the anti-inflammatory effects compared with those of commercial diclofenac tablet and diclofenac powder. Histological examinations were also carried out to investigate inflammatory changes and/or presence of micro-hemorrhagic lesions in the stomach in order to evaluate the mucoprotective potential of the natural gum.

Diclofenac sodium, a potent non-steroidal anti-inflammatory drug with short biological half-life (1–2 h), exhibits adverse effects such as gastrointestinal disturbances, peptic ulceration and gastrointestinal bleeding, with long-term use [16]. Formulation of diclofenac as controlled release microbeads using a polymer with mucoprotective properties will offer a means of delivering the drug in a sustained manner with reduced side effects.

2. Materials and methods

2.1. Methods

Diclofenac sodium was obtained from Fagron GmbH & Co (Barsbüttel, Germany) while sodium alginate and calcium acetate were obtained from Carl Roth GmbH & Co (Karlsruhe, Germany). Cissus gum was obtained from the stems of *C. pulpunea* from local farmers in Tose village, South West region of Nigeria. All other reagents were of analytical grade.

2.2. Extraction of cissus gum

Cissus gum was obtained from the stem of *C. pulpunea* using established procedure [12]. Briefly, cissus stem was soaked in chloroform water for 48 h to allow the gum to diffuse out of the stem. The gum was strained through a calico cloth to remove extraneous materials and then precipitated using absolute ethanol, washed with diethyl ether and then dried in hot air oven at 40 °C for 48 h. The died gum was pulverized, passed through a 150 μm sieve mesh sieve.

2.3. Preparation of microbeads

Diclofenac sodium microbeads were prepared by ionic gelation from gel blend of cissus gum and sodium alginate to obtain gum to alginate ratios of 1:1 and 2:1, with a total polymer concentration of 2.50% w/v. Appropriate quantity of diclofenac sodium was added such that the total polymer to drug ratio was 4:1. The resulting dispersion was extruded into calcium acetate solutions (5% w/v and 10% w/v) maintained under gentle agitation (300 and 400 rpm) using a syringe with 0.90 mm needle at a dropping rate of 2 mL/min. The formed beads were allowed 30 min curing time for cross-linking and then collected by decantation. The collected beads were washed with distilled water and dried for 24 h in hot air oven at 40 °C.

2.4. Characterization of microbeads

2.4.1. Size and morphology

The particle sizes of 100 microbeads were determined by using a computerized microscope fitted with a colored video (Letz Laborlux II, Wetzlar, Germany). The morphology and surface characteristics of the microbeads were analyzed using scanning electron microscopy (Hitachi Model S-2460N Taichung, Taiwan) at an accelerating voltage of 25 kV.

2.4.2. Entrapment efficiency

Diclofenac microbeads (50 mg) were crushed in a glass mortar and suspended in 10 mL of phosphate buffer, pH 6.8. After 24 h, the solution was filtered, appropriately diluted using phosphate buffer and analyzed spectrophotometrically at 274 nm using UV/Vis spectrophotometer (LAMBDA 12 Perkin Elmer GmbH, Ueberlingen, Germany). The drug entrapment efficiency (E) was calculated using the formula:

$$E\ (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

2.4.3. Drug release study

The *in vitro* drug release profile of the microbeads was determined in 900 mL of phosphate buffer, pH 6.8, maintained at 37 ± 0.5 °C, using the paddle method (USP XXI) rotated at a speed of 50 rpm. The absorbance was determined at 274 nm using UV/VIS spectrophotometer (LAMBDA 12 Perkin Elmer GmbH, Ueberlingen, Germany).

Data obtained from *in vitro* dissolution studies were fitted to zero order, first order, Higuchi, Hixon–Crowell, Korsemeyer–Peppas and Hopfenberg equations, to determine the kinetics and mechanism(s) of drug release from the microbeads [17,18]. The model of best fit was identified by comparing the values of correlation coefficients.

2.5. Experiment design

Factorial experimental design, which has been used as an efficient tool to obtain an appropriate mathematical model with minimum experiments, was used for the optimization of the formulation design [19,20] and to determine the effects of various formulation factors on the characteristics of the drug formulations [21]. A full 2^3 factorial design was performed (eight batches), using three independent process parameters, namely: cissus gum concentration (X_1), concentration of calcium acetate (X_2) and stirring speed (X_3) at two levels, high (+1) and low (−1). The individual and interactive effects of X_1, X_2 and X_3 on yield of microbeads, bead size, entrapment efficiency and dissolution time (t_{80}) were determined. Potential variables such as the ratio of total polymer to drug, curing time, needle size and dropping height were kept constant in the experimental design. The results obtained were then subjected to regression analysis using the software MINITAB (version 15, Pennsylvania, U.S.A). Response surface plots were obtained from the data.

2.6. In vivo *anti-inflammatory properties*

2.6.1. Experimental animals

Male albino rats weighing 125 ± 14 g were kept in eight groups of five in raised mesh bottom cages in environmentally controlled rooms (25 ± 2 °C, 12 h light and dark cycle). Animals were fed with standard pellets diet and water *ad libitum*. Animals were deprived of food 24 h before the experiment. Experimental protocols complied with the "Principle of Laboratory Animal Care" (NIH publication no 85-23) guidelines [22].

2.6.2. Induction of inflammation

Pedal inflammation in male albino rats was induced according to the method of Winter et al. [23]. Aqueous solution of 1% w/v carrageenan (0.2 mL) was administered into the right hind foot of the rats under the subplantar aponeurosis. Inflammation was quantified by measuring the paw size of the rats using a micrometer at the zero hour. Diclofenac (16 mg/kg) microbeads, commercial tablet or diclofenac powder was administered orally to each group 30 min after induction of inflammation, while water was administered to the control group. The paw size was measured at different time intervals and the percent swelling was determined using the equation [24]:

$$\%\ \text{Swelling} = \frac{C_t - C_o}{C_o} \times 100 \quad (2)$$

where C_o is the mean paw size at time zero and C_t is the mean paw size at time t.

The average paw swelling in the treated group was compared with that of control group and the percent inhibition was calculated using the formula:

$$\%\ \text{Inhibitation} = 1 - \frac{\%\ \text{swelling of treated group}}{\%\ \text{swelling of untreated group}} \times 100 \quad (3)$$

2.6.3. Histological examination

The animals were sacrificed 6 h after oral administration of the drug and their stomachs were dissected and examined visually for existing hemorrhagic lesions. The stomach tissues were placed in 10% buffered formaldehyde solution and fixed for 72 h. Small pieces of tissue specimen were collected in 10% phosphate buffer formalin at room temperature, rinsed in buffer, and dehydrated in a graded series of ethanol. These tissues were embedded in paraffin wax and sections of 5–6 μm in thickness were made and stained with hematoxylin and eosin for histological examination [25]. The structures of the gastric mucosa were examined microscopically and photographed with an Olympus BH-2 light microscope. Histological findings were evaluated and scored according to a system that has been described by McIntyre et al. [26]. The scoring system was as follows: Grade 0: no evidence of acute mucosal damage; Grade 1: mild acute inflammatory changes and/or presence of few micro-hemorrhagic loci; Grade 2: severe acute inflammatory changes and/or presence of several micro-hemorrhagic loci; Grade 3: acute inflammatory changes with evidence of severe erosions. Mean score of three independent investigators were taken. The damages was assessed using quantitative analysis based on parietal cell density, crypt depth and mucosal width [27].

2.7. Data analysis

Statistical analysis was carried out using the analysis of variance (ANOVA). Tukey's multiple comparison test was used to compare the difference between the formulations. At 95% confidence interval, probability, p values less than or equal to 0.05 were considered significant.

3. Results and discussions

3.1. Physicochemical and drug release properties of microbeads

The scanning electron micrographs of the microbeads are shown in Fig. 1. The result showed that small, discrete and spherical microbeads with rough surface morphology were obtained. The ranges of the three independent process parameters (X_1, X_2 and X_3) and the values of the percent yield, bead size and entrapment efficiency are presented in Table 1. The dissolution profiles of the microbeads are shown in Fig. 2 while the time taken for 80% drug release (t_{80}) determined from the plots are presented in Table 1. The results showed that the yield of the beads was between 88 and 97% while the beads size ranged from 1.22 ± 0.10 to 1.47 ± 0.14 mm and the entrapment efficiency was between 60.5 ± 5.3 and 91.5 ± 5.9%. The values of the yield of the beads and entrapment efficiency were higher while the particle size was lower than those previously reported for diclofenac microbeads using the same natural gum and zinc chloride as the chelating agent [12]. Thus, calcium acetate appears to be a better chelating agent for cissus bead than zinc chloride. Increasing the polymer to drug ratio to 4:1 also appears to improve the properties of the microbeads. The dissolution profiles from the microbeads showed that there was a lag time of about 2 h before the gradual release of diclofenac with time. The absence of initial "burst release" (a situation where 15% of the drug is released within the first hour) observed in the formulations is highly desirable for controlled release preparations. Burst release has been shown to lead to dose dumping, which could be of adverse pharmacological effect [28].

The values of the responses, which are indicative of the quantitative effects of the three variables on the properties of diclofenac microbeads, used to calculate individual and interaction coefficients are presented in Table 2. The ranking of the individual coefficients for the yield was in the order of $X_1 > X_2 > X_3$ showing that gum concentration had significantly ($p<0.05$) higher effect on yield of the formulation. The three variables, X_1, X_2 and X_3, showed negative effects on yield indicating that increasing the concentration of cissus gum in the polymer blend from 1:1 to 2:1, the concentration of calcium acetate from 5% w/v to 10% w/v and

Table 1
Factorial design for the formulation and the parameters used for the evaluation of diclofenac microbeads.

Batch	Coded levels			Real values			Response			
	X_1	X_2	X_3	X_1 cissus:alginate ratio	X_2 (%)	X_3 rpm	Yield (%)	Bead size (mm)	Entrapment (%)	t_{80} (h)
B_1	−1	−1	−1	1:1	5	300	96.3	1.33 ± 0.18	91.5 ± 5.9	14.0 ± 1.8
B_2	+1	−1	−1	2:1	5	300	92.8	1.37 ± 0.07	84.0 ± 3.4	12.2 ± 2.6
B_3	+1	+1	−1	2:1	10	300	93.8	1.47 ± 0.14	83.5 ± 4.7	16.6 ± 3.5
B_4	−1	+1	−1	1:1	10	300	95.1	1.34 ± 0.06	86.0 ± 6.4	14.4 ± 3.9
B_5	−1	−1	+1	1:1	5	400	97.0	1.22 ± 0.10	84.6 ± 3.7	15.2 ± 3.5
B_6	+1	−1	+1	2:1	5	400	92.3	1.30 ± 0.05	83.3 ± 3.9	13.4 ± 1.9
B_7	−1	+1	+1	1:1	10	400	95.0	1.24 ± 0.04	85.0 ± 6.4	16.2 ± 1.2
B_8	+1	+1	+1	2:1	10	400	87.9	1.35 ± 0.05	60.5 ± 5.3	7.2 ± 2.0

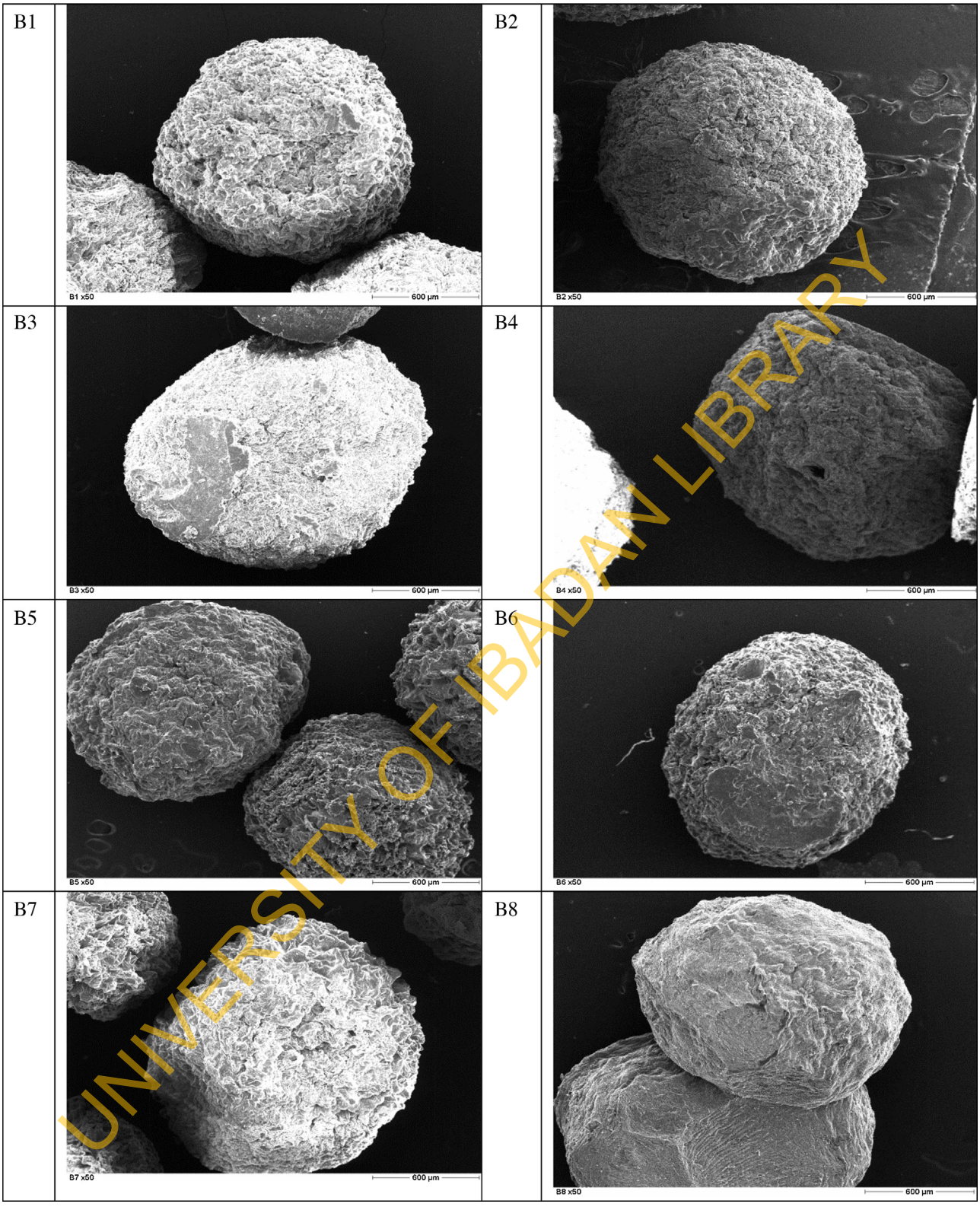

Fig. 1. Scanning electron micrographs of diclofenac sodium microbead formulations showing the morphology of the microbeads (Mag. ×50).

stirring speed from 300 rpm to 400 rpm gave a lower bead yield. On the other hand, the ranking of the coefficients on bead size was $X_3 > X_1 > X_2$, indicating that stirring speed and concentration of calcium acetate had significantly ($p<0.05$) higher influence on bead size. The individual coefficient values were positive for the influence of X_1 and X_2 on bead size but negative for X_3. This indicates that increase in cissus gum and calcium acetate concentrations resulted in the formation of larger beads whereas increase in stirring speed from 300 to 400 rpm resulted in the formation of smaller beads. Reduced stirring speed could result in increased inner phase viscosity, which in turn will promote coalescence resulting in the formation of larger beads. It has been reported that the surface

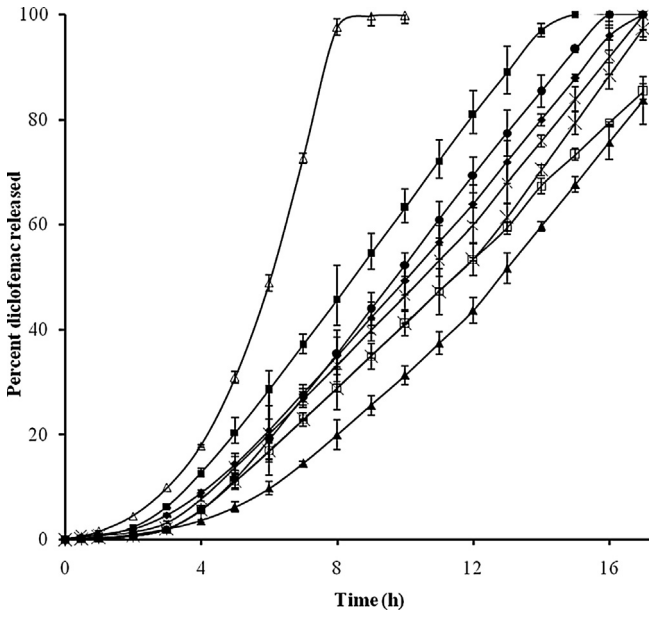

Fig. 2. The dissolution profiles of diclofenac sodium microbeads prepared using different concentration of cissus gum and chelating agent, and stirring speed: ♦, B1; ■, B2; ▲, B3; ×, B4; *, B5; ●, B6; □, B7; △, B8.

Table 2
Summary of the individual and interaction coefficients of the variables on the yield, bead size, entrapment efficiency and dissolution time (t_{80}) of diclofenac sodium microbeads.

Factor	Coefficient	Yield (%)	Bead size (mm)	Entrapment (%)	t_{80} (h)
X_1	Effect	−4.44	0.09	−8.83	−1.77
	p-Value	0.03	0.01	0.16	0.45
X_2	Effect	−1.36	0.05	−7.09	0.76
	p-Value	0.38	0.06	0.24	0.74
X_3	Effect	−1.14	−0.10	−8.16	−0.65
	p-Value	0.45	0.01	0.18	0.77
X_1X_2	Effect	−0.15	0.02	−2.28	−0.40
	p-Value	0.87	0.30	0.64	0.82
X_1X_3	Effect	−0.99	0.00	−1.98	−1.40
	p-Value	0.39	0.80	0.68	0.50
X_2X_3	Effect	−0.63	−0.01	−2.05	−1.25
	p-Value	0.53	0.63	0.01	0.54

area-to-volume ratios increases as bead size decreases [20]. Thus, water penetration into smaller particles may be faster due to the shorter distance from the surface to the center of the particle [29].

The ranking of the coefficients on entrapment efficiency was $X_1 > X_2 > X_3$, indicating that cissus gum concentration had the greatest influence on entrapment efficiency while stirring speed exhibited the lowest effect. The three factors showed negative effect on drug entrapment indicating that increasing the concentrations of cissus gum and calcium acetate, and stirring speed resulted in reduction in entrapment efficiency. Thus, microbeads with good entrapment efficiency could be obtained using lower concentration of cissus gum and calcium acetate, and lower stirring speed.

The ranking of the coefficients on t_{80} was in the order of $X_1 > X_2 > X_3$, indicating that concentration of cissus gum had the greatest influence on dissolution time, t_{80}. Factors X_1 and X_3 showed negative effect on dissolution time, indicating that increasing the concentration of cissus gum and stirring speed resulted in decreased dissolution time of the beads. On the other hand, the coefficient was positive for the influence of X_2 on t_{80} indicating that increasing the concentration of the chelating agent resulted in beads with slower dissolution rate. Consequently, higher amount of calcium acetate resulted in greater crosslinking which may retard the diffusion of drug out of the polymer [30]. Generally it was observed that the effect of concentration of cissus gum on yield was significantly higher ($p < 0.05$) than those of the other factors. On the other hand, the stirring speed had the most significant ($p < 0.05$) effect on bead size. This indicates that there is the need for careful selection of the formulation and processing variables employed in the formulation of microbeads using the natural gum.

The correlation coefficients of the kinetics of drug release from the microbeads, which were used as an indicator for best fit are presented in Table 3. The result showed that drug release from the microbead formulations fitted the Korsmeyer–Peppas model with correlation coefficients, $r^2 = 0.995$ in all cases. This indicates that drug release from the microbeads was controlled by a combination of diffusion and erosion mechanisms. This is consistent with previous reports on release kinetics of diclofenac and ibuprofen microbeads prepared using sodium alginate and some natural polymers [12,13].

The ranking of the interaction coefficients on bead size and entrapment efficiency was $X_1X_2 > X_2X_3 > X_1X_3$. This indicates that interaction between the concentration of cissus gum and calcium acetate had the greatest influence on size and entrapment efficiency of diclofenac microbeads. On the other hand, the ranking of the interaction coefficient on yield and dissolution time (t_{80}) was $X_1X_3 > X_2X_3 > X_1X_2$ indicating that the interaction between the concentration of gum and stirring speed had the largest influence on yield and dissolution time of the beads. It appears that gum concentration (X_1) interacted with all the variables to influence the bead properties. Formulation B5, which contained low concentration of cissus gum (1:1), low concentration of calcium acetate (5% w/v) and high stirring speed (400 rpm) produced bead with the smallest size and highest yield. Thus, formulation B5 was selected as the optimized microbead formulation, showing the highest yield (96.9%), smallest bead size (1.22 ± 0.10 mm) and a good balance between efficient entrapment ($84.6 \pm 3.7\%$) and sustained release of diclofenac with t_{80} value of 15.2 ± 3.5 h.

Surface plots generated for the graphical representation of the influence of two of the dependent variables, concentration of gum and concentration of calcium acetate on the properties of the microbeads are shown in Fig. 3. Generally, the steeper the slope,

Table 3
Correlation coefficients for the release of diclofenac microbeads using different release kinetic models.

Formulation	Zero order	First order	Higuchi	Hixson–Crowell	Korsmeyer		Hopfenberg
					r^2	n	
B1	0.9780	0.8851	0.8451	0.9624	*0.9924	1.7753	0.8610
B2	0.9798	0.7743	08818	*0.9827	0.9809	1.8562	0.8872
B3	0.9352	0.8167	0.7723	0.9292	*0.9952	2.0791	0.770
B4	0.9742	0.8212	0.8411	0.9579	*0.9826	1.8549	0.8605
B5	0.9611	0.8165	0.8149	0.9528	*0.9780	2.0974	0.8431
B6	0.9709	0.8079	0.8361	0.9583	*0.9769	2.3095	0.8088
B7	*0.9751	0.8818	0.8418	0.9587	0.9748	2.0672	0.8432
B8	0.9300	0.7654	0.7758	0.9367	*0.9896	2.0051	0.8157

* Highest correlation coefficient of drug release kinetics.

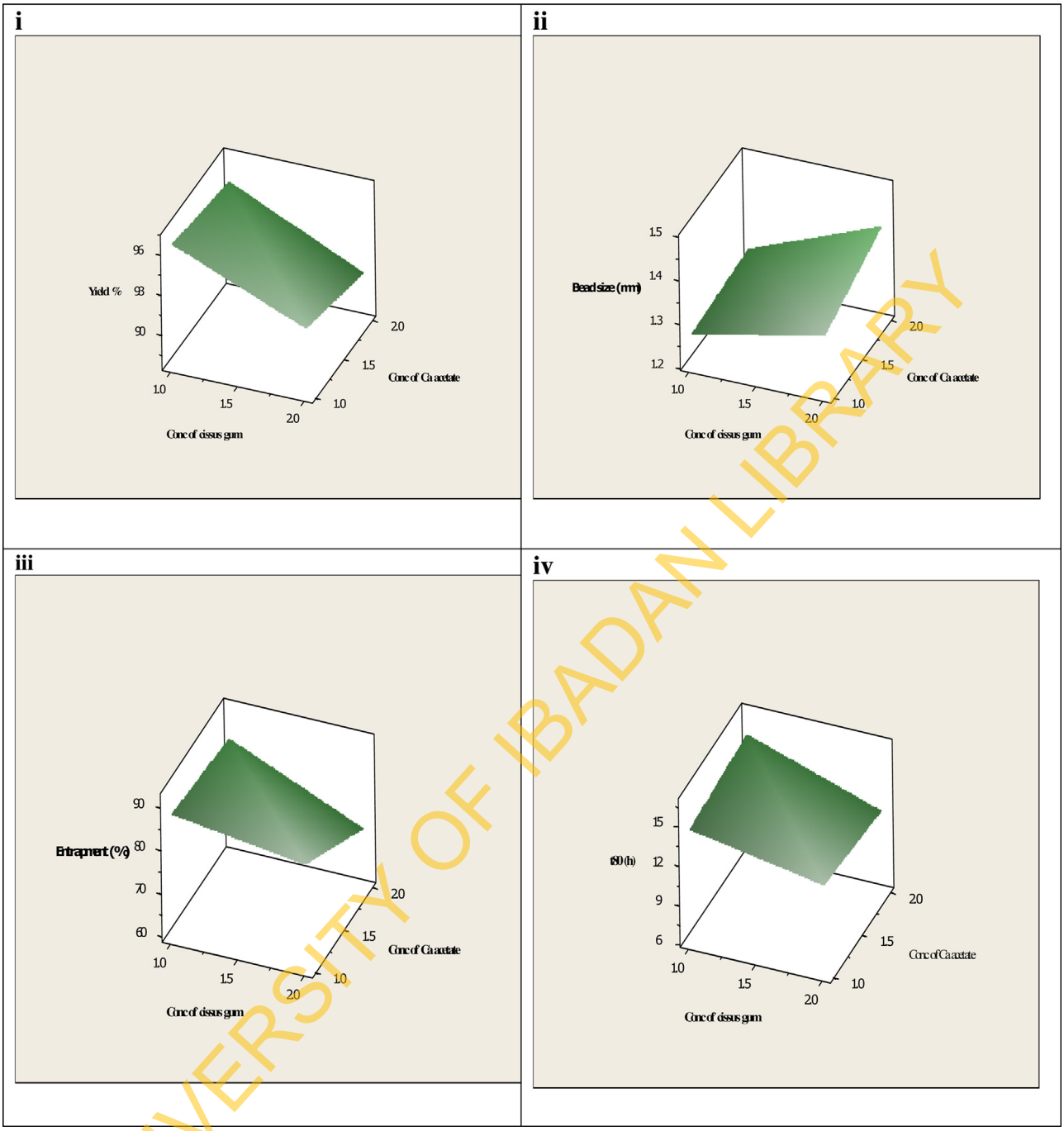

Fig. 3. The surface plots showing the effect of variables—concentration of cissus gum and chelating agent on (i) yield, (ii) bead size, (iii) entrapment and (iv) time for 80% drug release (t_{80}).

the stronger the interaction between the variables. The surface plots revealed that while the concentrations of cissus gum and calcium acetate interacted strongly to decrease the yield, entrapment efficiency and dissolution time, they however increased the size of the microbeads. Thus, there is the need for careful selection of formulation and process variables to obtain optimized microbeads.

3.2. In vivo *anti-inflammatory properties*

Carrageenan-induced inflammation has been used as *in vivo* model for assessing non-steroidal anti-inflammatory agents, NSAIDs [31,32]. Paw edema induced by carrageenan has been described as biphasic phase with the first phase being attributed to the release of histamine and serotonin, and the second phase attributed to the release of prostaglandins, protease and lysosome, which are sensitive to most clinically effective anti-inflammatory agents [33]. The changes in the paw size of rats with carrageenan induced edema at various time intervals are shown in Fig. 4. Carrageenan induced 22–27% increase in the size of rat paw immediately after injection and the paw size further increased to 23–45% after 1 h depending on whether drug was administered to the animals or not. There was a gradual reduction in paw size over time for treated animals but the paw size remained significantly higher ($p < 0.05$) in the control group. It can be observed that the microbead formulations exhibited slower onset of action compared with the

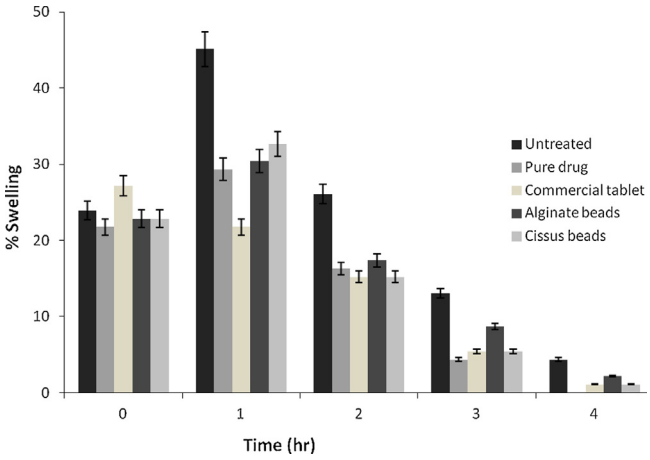

Fig. 4. Percent swelling of rat paw edema at various time intervals for untreated animals and animals administered with the pure drug, commercial tablets, alginate beads and cissus beads (mean ± SD, $n = 6$).

commercial tablet where the onset of action was immediately manifested by the gradual decrease in the swelling of the edema. This is consistent with the results of the drug release properties of the formulations. The ranking of the percent inhibition of swelling after 3 h was diclofenac powder > commercial tablet = cissus > alginate. Thus, diclofenac powder exhibited the highest percent inhibition of swelling while the microbeads containing cissus gum exhibited the same values as those of the commercial diclofenac tablets, and alginate beads exhibited the lowest inhibition.

Gastric ulcers still remain the main problem in clinical use of non-steroidal anti-inflammatory drugs which has limited their benefit in the treatment of many diseases. Macroscopical examination of the gastric mucosa of the experimental animals did not show marked signs of ulceration, which is expected since the administration of a single dose of the anti-inflammatory drug at relatively low dose (16 mg/kg) may not induce marked ulceration in the gastric mucosa [33]. The photomicrographs of the stomach mucosa of rats administered with the different formulations are presented in Fig. 5, while the parameters—parietal cell density, histological damage score, crypt depth and mucosal width which were used

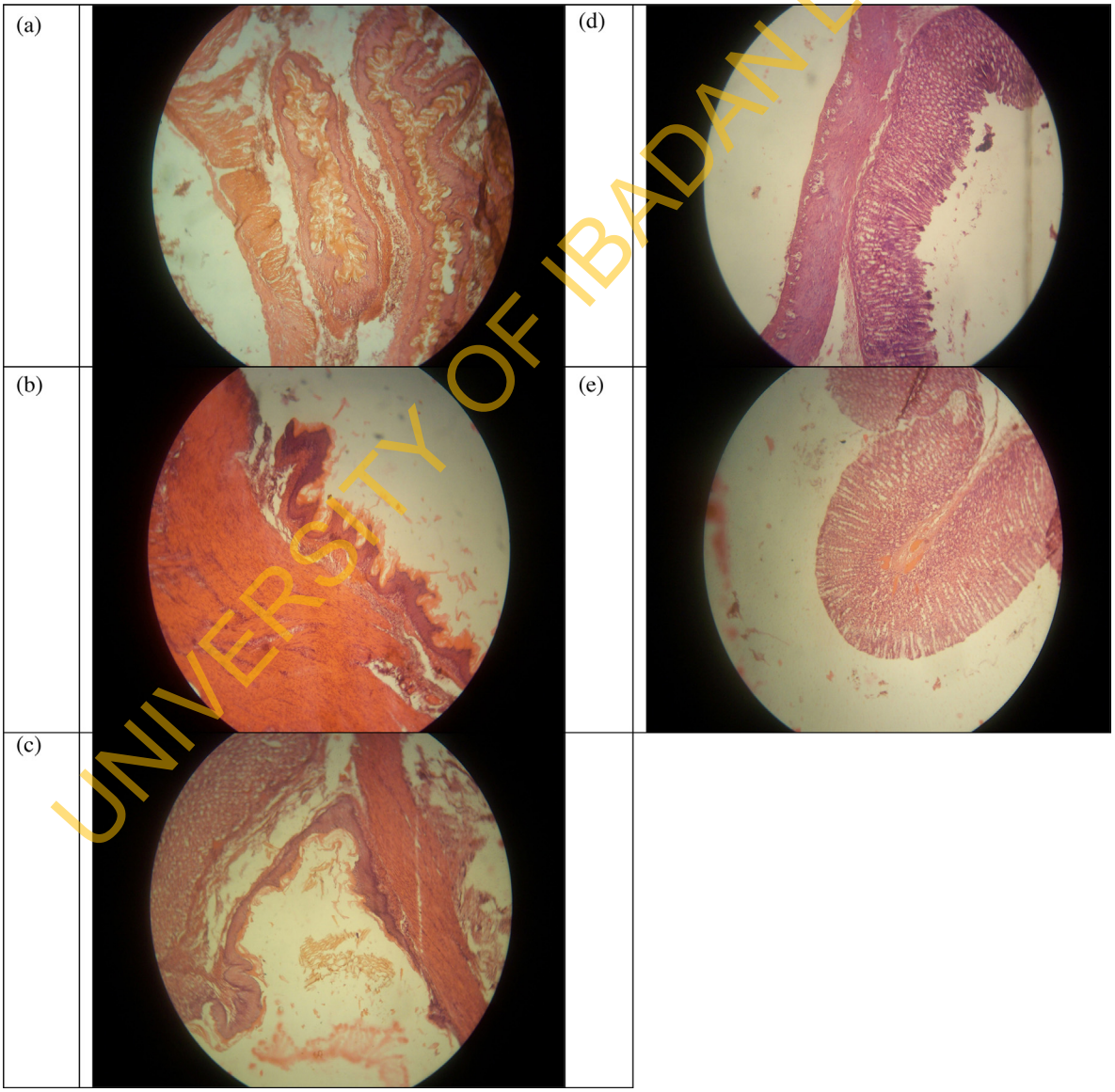

Fig. 5. Photomicrographs of the stomach sections of rats showing the histological damage: (a) control; (b) pure diclofenac; (c) commercial tablet; (d) alginate beads; (e) cissus beads (Mag. × 100).

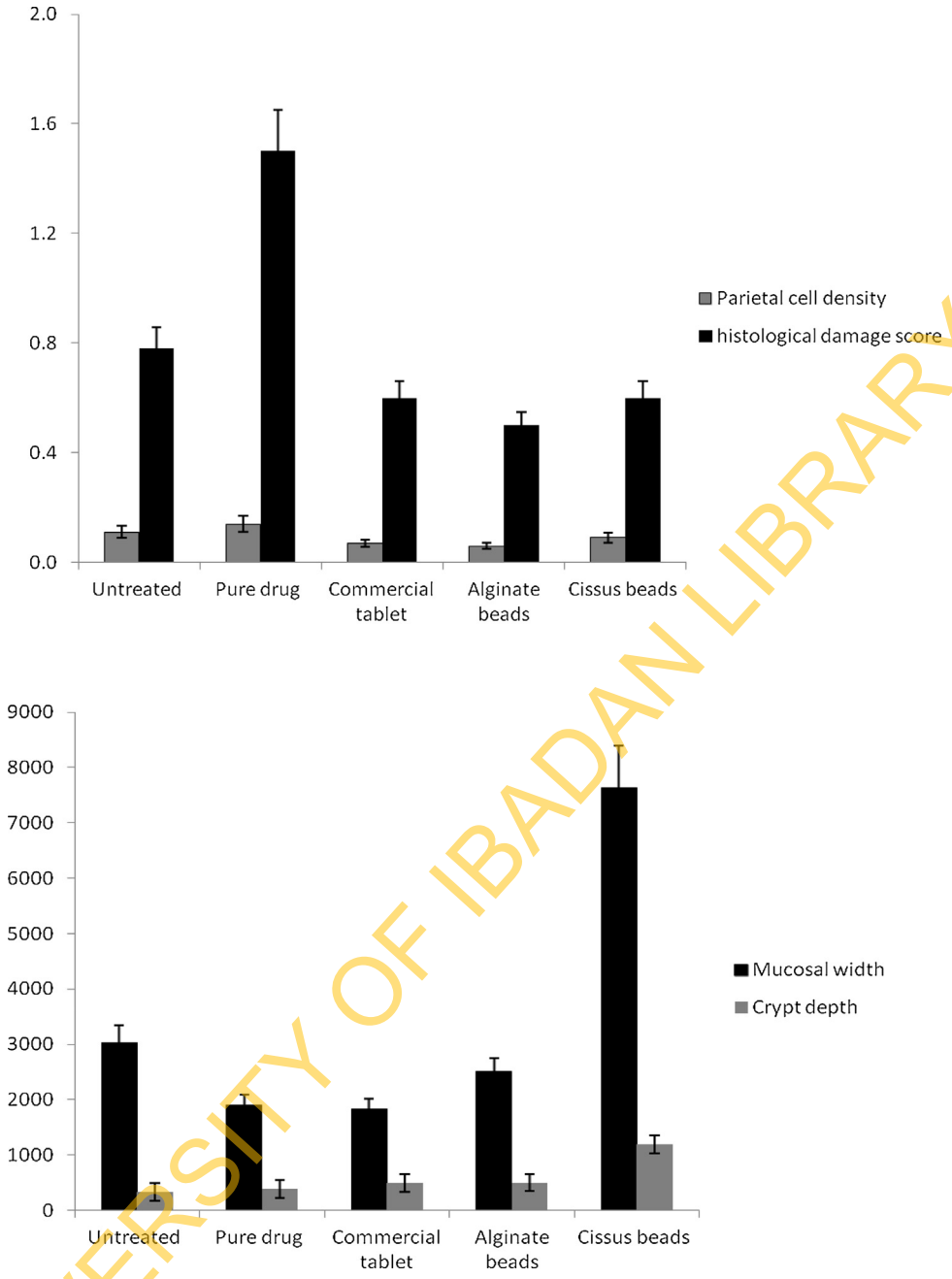

Fig. 6. Histological damage score for stomach section of rats according to the score system given in Section 2.1, parietal cell density (mm^{-2}), mucosal width (μm) and crypt depth (μm) for animals administered with pure diclofenac, commercial tablets, microbead formulations compared to the untreated group.

for the quantitative assessment of damages are presented in Fig. 6. Microscopical examination of the stomach however, revealed histological damage and the score was significantly higher in the group treated with diclofenac powder than for the animals receiving the microbead formulations and commercial tablet.

The parietal cell is responsible for secreting concentrated hydrochloric acid into the gastric lumen [34]. The gastric mucosa possesses a number of mechanisms that permit resistance to damage from its own secreted acid. Mucosal permeability to acid, active ion transport, blood flow, mucus secretion, epithelial restitution, and prostaglandin synthesis are among the multiple factors involved in gastric mucosal defense [35]. Non-steroidal anti-inflammatory drugs such as diclofenac sodium has been shown to cause gross mucosal damage by affecting the gastric mucosal defenses, making the mucosa more susceptible to the damaging effects of acid in the lumen [36]. Gastric acid secretion is known to be linearly related to the parietal cell density, and its effect on gastric mucosa is inversely related to the mucus cell population [37]. The results show that the rats administered with diclofenac powder had significantly higher ($p < 0.05$) parietal cell density when compared to those administered with commercial tablets and microbead formulations. Thus, the microbeads and commercial tablets appeared to reduce parietal cell density thereby attenuating acid secretion [38].

The result also showed that animals administered with cissus microbeads generally exhibited significantly higher ($p < 0.001$) mucosal width and crypt depth than those administered with the powdered drug, commercial tablet and alginate microbeads. On the other hand, there were no significant difference ($p > 0.05$) in the mucosal width and crypt depth of animals that were treated with

powdered drug and commercial tablet. High mucosal width and crypt depth have been shown to depict gastroprotective tendency of a drug [39]. Thus, cissus gum appeared to have gastroprotective effect, which is probably due to the formation of a hydrocolloid protective covering on the gastric mucosa leading to a reduction in the gastric ulcerative tendency of diclofenac. This non-specific mechanism has been similarly suggested for other drug delivery polymers in anti-inflammatory therapy [40]. In addition, cissus gum has been used in folkloric treatment of stomach pain and as a remedy for indigestion [14]. Studies has demonstrated the gastroprotective actions of the leaves and stem of two other species of cissus namely *Cissus sicyoides* L. and *Cissus quadrangularis* in non-steroidal anti-inflammatory drug induced gastric ulcer [41,42]. The extract of *C. quadrangularis* were shown to stimulate cell proliferation, gastric mucus synthesis and secretion in gastric ulcer model [43]. This supports the result obtained in the present study where cissus gum from *C. pulpunea* exhibited gastroprotective property. Thus, diclofenac microbeads containing the cissus gum did not induce hemorrhagic lesions and exhibited mucoprotective properties in rats suggesting their usefulness in the formulation of non steroidal anti-inflammatory drug with reduced side effects.

4. Conclusion

The factorial experimental design has been used for the careful selection of various processing and formulation variables required to produce optimized diclofenac microbeads. The results revealed that the concentrations of cissus gum had the greatest influence on yield, entrapment efficiency and drug release properties of diclofenac microbeads while stirring speed had the greatest influence on bead size as was evident by the magnitude of their individual coefficients. Optimized diclofenac microbeads were prepared using polymer blend of cissus gum and alginate at a ratio of 1:1 with 5% w/v calcium acetate as cross-linking agent at stirring speed of 400 rpm. *In vivo* studies revealed that cissus gum-based diclofenac microbeads were as effective as commercial diclofenac tablet in reducing carrageenan induced paw inflammation in rats. Furthermore, cissus containing microbeads significantly ($p < 0.05$) induced lower parietal cell density and higher mucosal width and crypt depth than powdered drug and commercial tablet suggesting the mucoprotective property of the gum. Thus, cissus gum is suitable as a versatile excipient for the delivery of anti-inflammatory drug with the possibility of sustaining the drug release, especially the absence of a burst release, which could be very beneficial in NSAIDs therapy.

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