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Amelioration of Beta-Sitosterol Solid Lipid Nanospheres On Indomethacin Induced Gastric Ulcer in Zebrafish Model

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ABSTRACT:

Gastric ulcer is a deep, painful inflammation in the stomach lining that can even lead to bleeding. Beta-sitosterol is a phytosterol having anti-inflammatory activity. The study focuses on the Amelioration of Beta-Sitosterol Solid Lipid Nano-spheres on Indomethacin induced gastric ulcers in the Zebrafish model. The zebrafish is an appealing model organism for investigating anti-ulcer activity and their interactions. Zebrafish also promises to be a good tool to study anti-oxidant and anti-inflammatory activities. The mechanism behind the study is the binding of beta-sitosterol with the 3whf PGE2 protein receptor which synergizes the synthesis of PG resulting in anti-inflammatory activity.

Keywords: beta-sitosterol, zebrafish, gastritis, anti-ulcer, anti-inflammatory

1. Introduction

Gastric ulcers are defined as perforations in the mucosa lining the stomach that penetrate through the muscularis mucosa and are more than 5 mm in diameter. These happen when the stomach's defences are weakened, altering the gastric mucosa and causing erosion and ulceration. Treatment options for the condition depend on what caused the ulcer, although both prevention and treatment are possible. Prostaglandins, mucus, growth hormones, and an adequate blood supply are necessary for the stomach mucosa to retain its protective covering. Chemicals that harm this barrier include alcohol, tobacco, hydrochloric acid, ischemia,

NSAIDs, hypoxia, and H. pylori infection [1,2](B. J. Marshall et al. 1985; B. Marshall and Warren 1984).
The most common sign of peptic ulcers, lesions on the stomach lining and upper small intestine [3](Kellerman RD 2020), is stomach discomfort. Long-term NSAID use and H. pylori infection are the main causes of them. Although they can exacerbate symptoms, stress and spicy foods do not cause peptic ulcers [4,5](Sloan JM, n.d.; Li et al. 2010). Peptic ulcer disease (PUD) is still a major global health concern that causes a high number of hospital admissions. The detrimental effects of stomach acid and pepsin are outweighed by defensive mechanisms including mucus and bicarbonate production in PUD [6,7](Wang 2011; Aro et al. 2006).

2. Materials and Methods

Molecular Docking
Docking was performed by downloading Drug moiety and receptor protein from PDB, the program was done using Auto-dock and MGI tools and docking score was obtained and evaluated [8-11](Mukherjee, Ray, and Thakur 2009; Kedare and Singh 2011; WATANABE et al. 2012; Zatorska et al. 2020).

Solid Lipid Nanospheres (SLNs) Prepared
Solvent diffusion and hot homogenization, with some changes to a previously published methodology, was used to create β-sitosterol SLNs. The selection of solid lipids was based on solubility. 2g of soy lecithin and 3g of poloxamer were employed in the formulation. At 70°C, both were melted, and 3g of β-sitosterol was added, stirring constantly until it was completely dissolved. A 3% w/v Tween 80 solution was made separately in 10 millilitres of water. To create a homogeneous dispersion, the aqueous phase was then homogenized into the organic phase for two to six minutes at 10,000 rpm. After adding an extra 10 mL of water, the mixture was constantly stirred in an ice bath for 1-4 hours at 1600 rpm. For additional storage, the resultant SLNs were kept in a refrigerator [8-11](Mukherjee, Ray, and Thakur 2009; Kedare and Singh 2011; WATANABE et al. 2012; Zatorska et al. 2020).

Entrapment Efficiency (EE)
A modified technique from Rahman et al. was used to determine the loading capacity (LC) and entrapment efficiency (EE) of medicines in SLNs. After ultracentrifuging a 2 mL dispersion aliquot of SLNs, the supernatant was disposed of. To extract the medication, the pellet containing SLNs was subjected to 15 minutes of sonication. After that, the medication was gathered in the mobile phase and measured by HPLC, or high-performance liquid chromatography. The following equation was used to estimate IC and EE [8-11] (Mukherjee, Ray, and Thakur 2009; Kedare and Singh 2011; WATANABE et al. 2012; Zatorska et al. 2020)..

$$LC\left(\%\right) = \frac{Entrapped\ \ Drug}{Nanoparticles\ \ weight} \times 100$$

$$\mathrm{EE}\,\% = \frac{Total\ \ quantity\ \ of\ \beta - \text{sitosterol} - Amount\ \ of\ \beta - \text{sitosterol in total supernaent}}{Amount\ \ of\ \beta - \text{sitosterol}} \times 100$$

Figure 1: Entrapment Efficiency (EE)

Zeta-Potential

The zeta potential of the optimized nano emulsion system was measured using a zeta-sizer. Each sample (100 microliters) was diluted with distilled H2O at a ratio of 1:100 before analysis [8-11] (Mukherjee, Ray, and Thakur 2009; Kedare and Singh 2011; WATANABE et al. 2012; Zatorska et al. 2020).

In Vitro Product Release

To ascertain the release pattern, the in vitro drug release of β-sitosterol-SLNs was studied using phosphate buffer saline (PBS; pH 7.4) to dissolve the SLNs. The mixture was then shaken for 24 hours at 100 rpm and placed inside a dialysis bag. For the experiment, a 2.5 mg SLN dispersion was put into the dialysis bag. At 37°C, 0.5 mL samples were obtained on a regular basis, and each time, the same volume of new medium was introduced. The samples were examined using UV spectroscopy in order to determine the percentage of medication release. Next, based on varied time intervals, the release data were matched to many mathematical models [8-11] (Mukherjee, Ray, and Thakur 2009; Kedare and Singh 2011; WATANABE et al. 2012; Zatorska et al. 2020).

Pharmacological Studies

Antioxidant Assays

The 2,2-Diphenyl-1-Picrylhydrazyl Assay, or DPPH Assay, was applied to assess the radical-scavenging potential for multiple extracts. This experiment assesses how adding an antioxidant reduces the absorbance of the DPPH solution at 517 nm. As a reference, 10 mg/mL of ascorbic acid in DMSO was employed. An antioxidant turns yellow when it scavenges the red, stable free radical DPPH. The interaction between DPPH and an antioxidant (H-A) is as follows:

(DPPH) + (H-A) = DPPH + (A)

Antioxidants convert DPPH to DPPH-H when they interact with it, lowering absorbance. The level of discoloration indicates how well extracts or antioxidant compounds scavenge hydrogen.

Preparation of the Reagent: 100 mL of ethanol was used to dissolve 4 mg of DPPH for the reaction.

Operational protocol

2.96 mL of 0.1 mM DPPH solution was added to different volumes (2-20 µL) of plant extracts diluted with DMSO to achieve a final volume of 40 µL. For twenty minutes, the reaction mixture was allowed to stand at room temperature in the dark. The absorbance was measured at 517 nm after 20 minutes. Moreover, there was a control that contained 3 mL of DPPH. Using the following formula, the percentage of radical scavenging activity (% RSA) in the sample was calculated:

% RSA = [(Abs control - Abs sample) / Abs control] x 100

RSA stands for Radical Scavenging Activity. Abs sample refers to the absorbance of the DPPH radical with the sample, and Abs standard refers to the absorbance of the DPPH radical with ethanol [12-16](Vasileva et al. 2017; Gryszczynska et al. 2015; Cuzzocrea et al. 2005; Oh, Kim, and Jeong 2011; Wong et al. 2014).

Protein Denaturation Method

Two millilitres (mL) of phosphate-buffered saline (PBS, pH 6.4) and 0.2 mL of fresh hen egg albumin (from an egg) were combined with the medication used in the experiment at different

concentrations (0.2, 0.6, and 0.8 mg/mL). The control was an equivalent volume of double-distilled water. After incubating at 37±2°C for 15 minutes in a BOD incubator, the mixture was heated to 70°C for five minutes. After cooling, the vehicle was used as a blank to measure the absorbance at about 660 nm [12-16] (Vasileva et al. 2017; Gryszczynska et al. 2015; Cuzzocrea et al. 2005; Oh, Kim, and Jeong 2011; Wong et al. 2014).
%inhibition = 100* (Vt/Vc-1)
where, Vt = Absorbance of the sample, Vc = Absorbance of the control

In-vivo Screening Method
Over seventy percent of the genes involving humans and zebrafish are similar, and eighty-four percent of the human genes associated with human disease also occur in zebrafish. In the experimental fish facility at SRM University, Kattankulathur, an adult wild-type zebrafish weighing between 0.4 g and 0.8 g was reared. The fish were divided into five groups, each of which contained six zebrafish with 1l of fresh water and an aquarium oxygen air pump motor that delivers additional oxygen. The fish were then isolated for a week. Fish were fed commercial feed twice daily and kept at a temperature of 26-28.5 °C. Further food and environmental modifications were done and observed.

Indomethacin-induced gastritis: Commercial feeding was halted one day before the trial. Aside from the control group, all four groups began receiving oral inductions of the medication indomethacin. Prednisolone (0.1 mg/g) was administered orally to the Standard group, 0.6 mg of Beta-sitosterol was administered orally to the High dose group, and 0.3 mg of Beta-sitosterol was administered orally to the Low dose group. The experiment was repeated six days later with the afflicted group, and on the seventh day, the fishes were sent for histopathological evaluation [12-16] (Vasileva et al. 2017; Gryszczynska et al. 2015; Cuzzocrea et al. 2005; Oh, Kim, and Jeong 2011; Wong et al. 2014).

3. Results and Discussion

Docking Result
Fig:2 & 3 explains In-silico docking was performed with the target protein 3wfh PGE2 and it revealed that it has a good binding affinity with the drug which is showed by binding energy -6.53 kgmol-1.

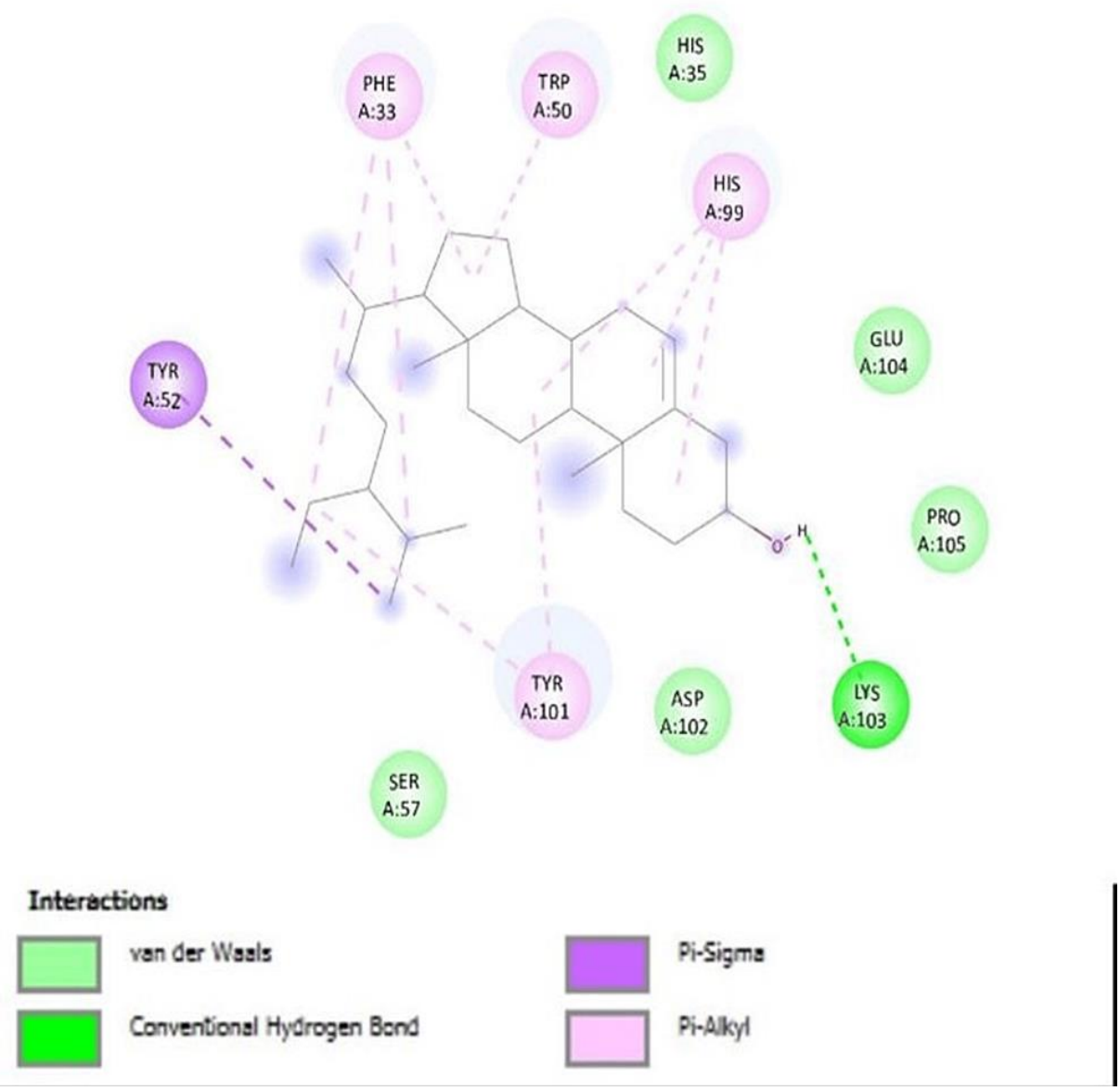

Figure 2: Molecular Docking

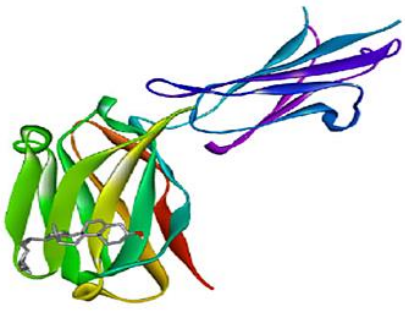

Figure 3: Dock Binding

Percentage Entrapment Efficiency

In Table 1: The entrapment efficiency was calculated for Beta-sitosterol. The maximum entrapped efficiency was absorbed as 92%.

S.No	Formulation	Entrapment Efficiency (%)
1	Beta-Sitosterol solid lipid nano spere (SLNs)	92

Table 1: Entrapment efficiency of Beta-sitosterol

Particle Size and Zeta Potential Analysis

In Table 2: Particle size and zeta potential analysis of the optimized formulation were conducted using a zeta-sizer (DTS Version 5.03, Malvern) employing the light scattering method. The vesicle diameter was found to be 615.5 nm. The zeta potential was found to be -0.1 mV. I

Formulation	Zeta-Average Size	PDI
Beta- Sitosterol	615.5nm	0.751

Table 2: Zeta Potential

S.NO	TIME	CUMULATIVE % RELEASE
1.	5 Mins	14
2.	15 Mins	38
3.	30 Mins	53
4.	45 Mins	75
5.	1 Hour	96
	N=3+S.D N=3-S. D	

Table 3: Cumulative % In-vitro release with respect to time

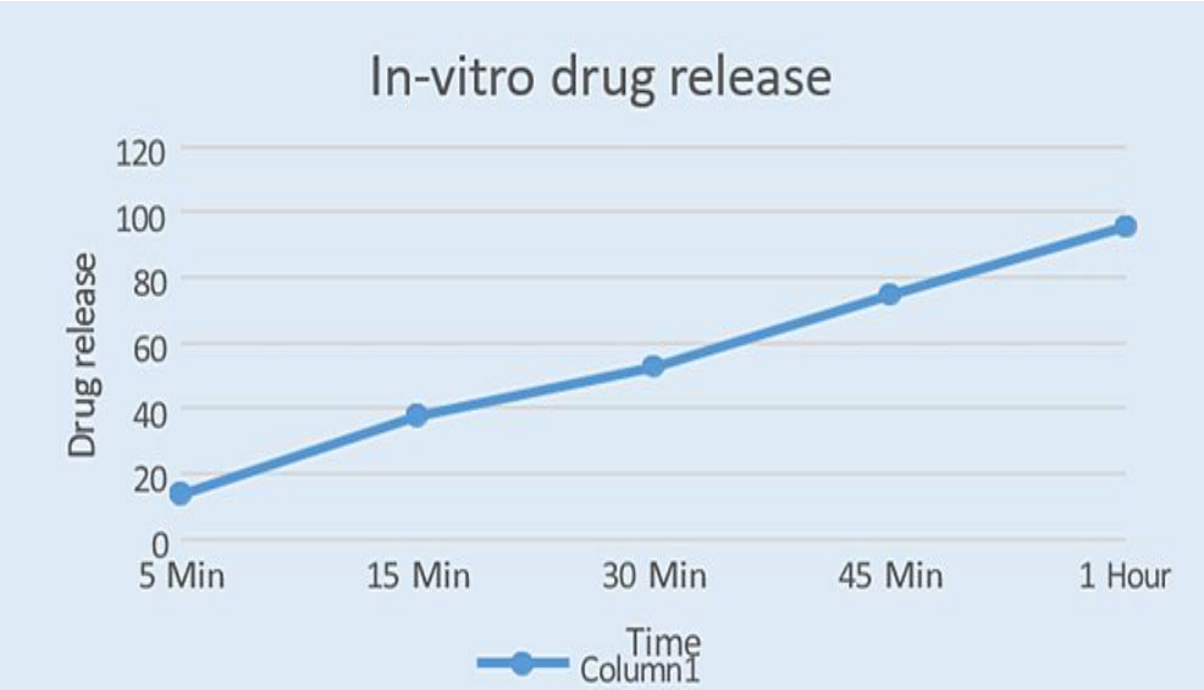

Figure 4: In- Vitro Drug Release graph with respect to Time

Pharmacology Studies

Concentration (µg/ml)	Absorbance
o	0.557
0.2	0.485
0.5	0.346
0.8	0.227

Table 4: UV-Absorption (DPPH Assay)

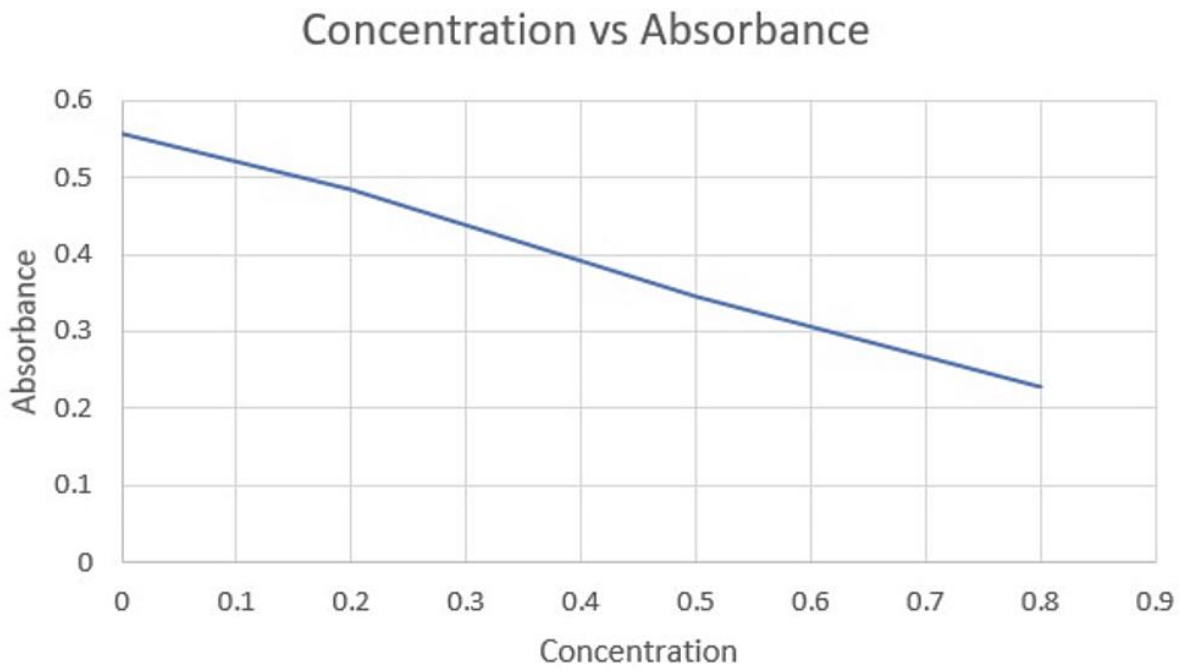

Figure 5: Concentration Vs Absorbance (DPPH ASSAY)

Protein Denaturation

Concentration (µg/ml)	% inhibition (Std-Diclofenac)	% inhibition (Test-Beta-sitosterol)	Viscosity(cp)
Control	-	-	-
200	2.7	2.5	0.73
400	5.2	4.9	0.76
600	8.1	7.8	0.79
800	10.3	10.1	0.82
1000	12.5	12.4	0.85

Table 5: Protein Denaturation

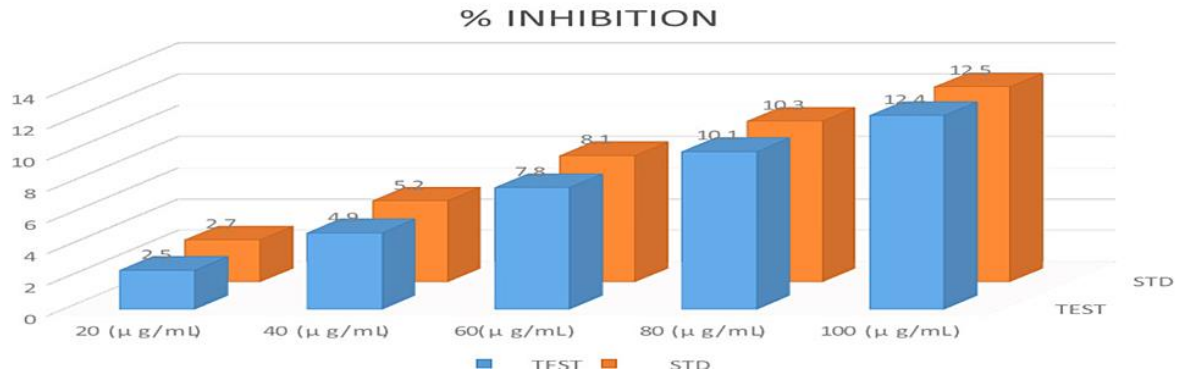

Figure 6: Percentage Inhibition (X-Axis Represents Concentration and Y-Axis Represents % Inhibition)

In-vivo Study Histopathology

The pictures evidence the histopathological changes in the five different groups with their perforation and ulcerative pattern as follows in which the control group has a well developed histological characters and the disease group has a poorly developed or highly perforated histological character, whereas standard shows as expected growth pattern, low dose and high dose show a decreased in perforation and ulcerative index respectively. Fig (7-11) show the histopathological evaluation in different groups

GROUP	ULCERATIVE SCORE
CONTROL	0
DISEASE	3
LOW DOSE	2
HIGH DOSE	1
STANDARD	0.5

Table 6: Ulcerative Score

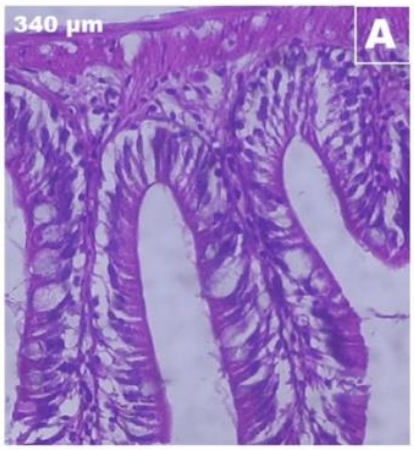

Figure 7: Gastric section form normal zebrafish showing normal mucosa and sub-mucosa in control group (A)

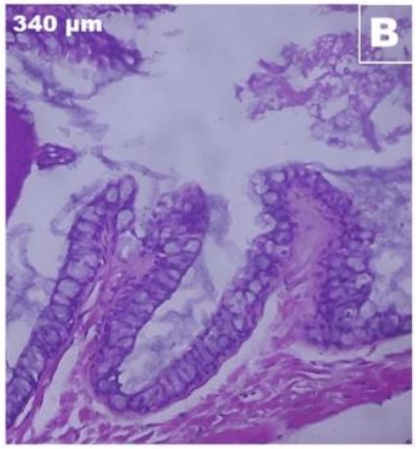

Figure 8: Gastric section form untreated zebrafish showing ulceration of the mucosal cells associated with inflammatory changes and necrosis in diseased group (B)

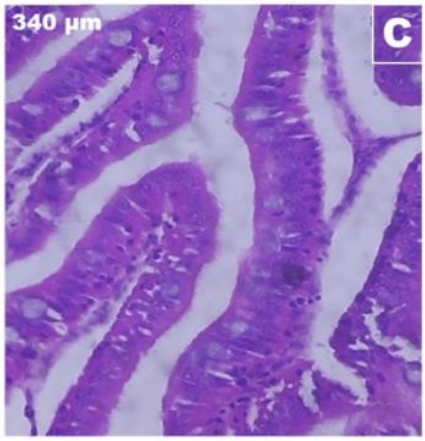

Figure 9: Gastric section form Beta-sitosterol drug treated zebrafish showing a decreased in perforation and ulceration of the mucosal cells in high dose group (C)

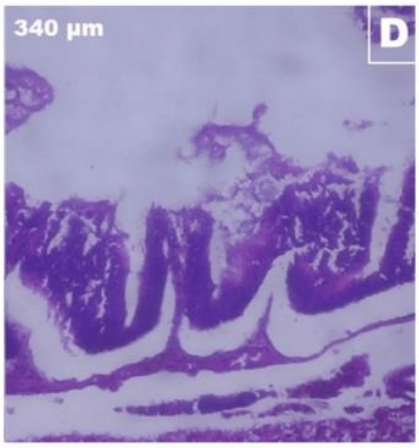

Figure 10: Gastric section form Beta-sitosterol drug treated zebrafish showing a decreased in perforation and ulceration of the mucosal cells in low dose group (D)

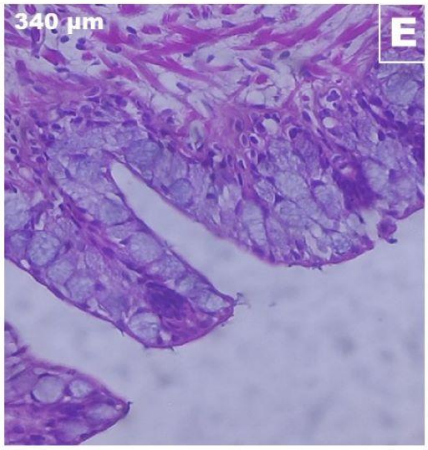

Figure 11: Gastric section form Prednisolone showing expected ulceration growth pattern of the mucosal cells in standard group (E)

4. Discussion

Using zebrafish model, the study investigated the curative effects of beta-sitosterol solid lipid nanospheres (SLNs) to mitigate indomethacin-induced stomach ulcers. Key findings comprise of: The 3wfh PGE2 protein receptor displayed an enormous binding affinity (-6.53 kcal/mol) for beta-sitosterol, revealing possible anti-inflammatory efficacy through prostaglandin production enforcement and SLNs with a mean particle size of 615.5 nm and a high entrapment

capacity of 92% were effectively produced. In vitro, this formulation showed sustained drug release, suggesting that the therapeutic impact might last longer.
Pharmacological Activities concludes that amino acid denaturation experiment, beta-sitosterol had excellent anti-inflammatory effects and strong antioxidant activity, indicating its potential as a remedy for stomach ulcers. Beta-sitosterol SLNs at different levels in the zebrafish model showed diminished ulcerative index in contrast with the disease group, revealing protective effects on the gastrointestinal mucosa.

5. Conclusions

In the present study, beta-sitosterol is the drug of choice due to its identified anti-inflammatory and anti-oxidant property which leads a way in the treatment of gastric ulcer. After receiving a binding score of -6.53Kgmol-1 from the docking study, which demonstrated the drug's effectiveness in binding to the receptor site, which lead to further formulation of beta-sitosterol solid lipid nanospheres. The entrapment efficiency and in-vitro drug release evidences the pharmacokinetic and pharmacodynamic parameters of the beta-sitosterol solid lipid nano spheres and eventually ensures its physical properties. The percentage entrapment efficiency was found to be 92%. The vesicles and zeta potential were determined to be 615.5nm. The invitro drug release evidences that drug release increases as time increases. Pharmacological invitro study using DPPH assay demonstrates that as the concentration rises, the absorbance falls which shows the predominant anti-oxidant activity. The formulation was found to have anti-inflammatory property recognized by protein denaturation, in this case, the viscosity increases with reduction in concentration. When compared to the disease group, pharmacological In-vivo investigation demonstrates that the ulcer score for the medicine treated is lower. Hence the study concludes that the beta-sitosterol solid lipid nano spheres had a protective effect on indomethacin induced gastric ulcer in zebrafish.

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6. References

1. Aro, Pertti, Tom Storskrubb, Jukka Ronkainen, Elisabeth Bolling-Sternevald, Lars Engstrand, Michael Vieth, Manfred Stolte, Nicholas J. Talley, and Lars Agréus. 2006. "Peptic Ulcer Disease in a General Adult Population." American Journal of Epidemiology 163 (11): 1025–34. https://doi.org/10.1093/aje/kwj129.
2. Cuzzocrea, Salvatore, Emanuela Mazzon, Rosanna di Paola, Tiziana Genovese, Carmelo Muià, Achille P. Caputi, and Daniela Salvemini. 2005. "Synergistic Interaction between Methotrexate and a Superoxide Dismutase Mimetic: Pharmacologic and Potential Clinical Significance." Arthritis & Rheumatism 52 (12): 3755–60. https://doi.org/10.1002/art.21480.
3. Gryszczynska, A., P. L. Mikolajczak, P. Grzadzielski, P. Zakowicz, M. Szulc, E. Kaminska, W. Buchwald, et al. 2015. "FOC8-3COMPARISON OF EXTRACTS FROM ROOT OF RHODIOLA ROSEA AND RHODIOLA KIRILOWII INHIBITORY ACTION ON ALCOHOL TOLERANCE DEVELOPMENT IN RATS." Alcohol and Alcoholism 50 (suppl 1): i44.1-i44. https://doi.org/10.1093/alcalc/agv079.33.
4. Kedare, Sagar B., and R. P. Singh. 2011. "Genesis and Development of DPPH Method of Antioxidant Assay." Journal of Food Science and Technology 48 (4): 412–22. https://doi.org/10.1007/s13197-011-0251-1.

5. Kellerman RD. 2020. Gastritis and Peptic Ulcer Disease. In: Conn's Current Therapy.
6. Li, Zhaoshen, Duowu Zou, Xiuqiang Ma, Jie Chen, Xingang Shi, Yanfang Gong, Xiaohua Man, et al. 2010. "Epidemiology of Peptic Ulcer Disease: Endoscopic Results of the Systematic Investigation of Gastrointestinal Disease in China." American Journal of Gastroenterology 105 (12): 2570–77. https://doi.org/10.1038/ajg.2010.324.
7. Marshall, Barry J., David B. McGechie, Peter A. Rogers, and Ross J. Clancy. 1985. "Pyloric Campylobacter Infection and Gastroduodenal Disease." Medical Journal of Australia 142 (8): 439–44. https://doi.org/10.5694/j.1326-5377.1985.tb113444.x.
8. Marshall, BarryJ, and J.Robin Warren. 1984. "UNIDENTIFIED CURVED BACILLI IN THE STOMACH OF PATIENTS WITH GASTRITIS AND PEPTIC ULCERATION." The Lancet 323 (8390): 1311–15. https://doi.org/10.1016/S0140-6736(84)91816-6.
9. Mukherjee, S, S Ray, and RS Thakur. 2009. "Solid Lipid Nanoparticles: A Modern Formulation Approach in Drug Delivery System." Indian Journal of Pharmaceutical Sciences 71 (4): 349. https://doi.org/10.4103/0250-474X.57282.
10. Oh, Hyun-A, Hyung-Min Kim, and Hyun-Ja Jeong. 2011. "Distinct Effects of Imperatorin on Allergic Rhinitis: Imperatorin Inhibits Caspase-1 Activity In Vivo and In Vitro." Journal of Pharmacology and Experimental Therapeutics 339 (1): 72–81. https://doi.org/10.1124/jpet.111.184275.
11. Sloan JM. n.d. "Acute Haemorrahagic Gastritis and Acute Infective Gastritis; Gastritis Caused by Physical Agents and Corrosives; Uraemic Gastritis. In: Whitehead R, Ed. Gastrointestinal and Oesophageal Pathology."
12. Vasileva, Liliya V., Kremena E. Saracheva, Mariya V. Ivanovska, Atanaska P. Petrova, Emir Sucouglu, Mariana A. Murdjeva, and Damyanka P. Getova-Spasova. 2017. "Beneficial Effect of Chronic Treatment with Extracts from Rhodiola Rosea L. and Curcuma Longa L. on the Immunoreactivity of Animals Subjected to a Chronic Mild Stress Model." Folia Medica 59 (4): 443–53. https://doi.org/10.1515/folmed-2017-0046.
13. Wang, Fu-Wei. 2011. "Prevalence and Risk Factors of Asymptomatic Peptic Ulcer Disease in Taiwan." World Journal of Gastroenterology 17 (9): 1199. https://doi.org/10.3748/wjg.v17.i9.1199.
14. WATANABE, Hiroko, Chie AKABOSHI, Haruko SEKIDO, Kouki TANAKA, Kazuko TANAKA, and Naoki SHIMOJO. 2012. "Denaturation of Egg Antigens by Cooking." Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi) 53 (2): 98–104. https://doi.org/10.3358/shokueishi.53.98.
15. Wong, Hoi-Shan, Ji-Hang Chen, Pou-Kuan Leong, Hoi-Yan Leung, Wing-Man Chan, and Kam-Ming Ko. 2014. "β-Sitosterol Protects against Carbon Tetrachloride Hepatotoxicity but Not Gentamicin Nephrotoxicity in Rats via the Induction of Mitochondrial Glutathione Redox Cycling." Molecules 19 (11): 17649–62. https://doi.org/10.3390/molecules191117649.
16. Zatorska, Maria, Grzegorz Łazarski, Urszula Maziarz, Natalia Wilkosz, Tomoko Honda, Shin-ichi Yusa, Jan Bednar, Dorota Jamróz, and Mariusz Kepczynski. 2020. "Drug-Loading Capacity of Polylactide-Based Micro- and Nanoparticles – Experimental and Molecular Modeling Study." International Journal of Pharmaceutics 591 (December):120031. https://doi.org/10.1016/j.ijpharm.2020.120031.