RESEARCH ARTICLE

In-vitro, In-vivo Study of *Jatropha curcas* Leaves Extract, and Preparation of a Nanoemulsion by a Low-energy/Solvent-free Method, *In-silico* Study of β-Sitosterol in Ulcerative Colitis

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Received: 15th June, 2023; Revised: 01st January, 2024; Accepted: 18th February, 2024; Available Online: 25th March, 2024

ABSTRACT

Introduction: Ulcerative colitis (UC) is characterized by recurring colon inflammation, leading to severe symptoms and decreased quality of life. *Jatropha curcas*, a traditional remedy for UC, particularly its active component β-sitosterol, is investigated here for its potential therapeutic benefits.

Methods: The study evaluated *J. curcas* leaf extract's effects on RAW 264.7 cell lines *in-vitro*, demonstrating a significant reduction in IL-6 production and promising anti-UC properties. *In-vivo* experiments on BALB/C mice with DSS-induced colitis confirmed dose-dependent reductions in disease severity, colon tissue damage, and pro-inflammatory cytokine levels. Additionally, a solvent-free nanoemulsion of *J. curcas* leaf extract was developed, exhibiting enhanced stability, encapsulation efficiency, and sustained release of active components, offering a promising drug delivery strategy for UC therapy. This interdisciplinary research contributes valuable insights into the therapeutic potential of *J. curcas* leaf extract and its nanoemulsion for UC treatment. Molecular docking simulations suggest strong binding affinities between β-sitosterol and inflammatory mediators, shedding light on its anti-inflammatory mechanisms. Pharmacokinetic and toxicity profiling further support the development of *J. curcas*-based UC therapeutics, laying a solid foundation for future studies and clinical applications.

Keywords: Ulcerative colitis, *Jatropha curcas*, β-sitosterol, Nanoemulsion, Anti-inflammatory, Therapeutic, *In-vitro, In-vivo,* Molecular docking, Drug delivery.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.38

How to cite this article: Gayathiri K, Chitra V. *In-vitro, In-vivo* Study of *Jatropha curcas* Leaves Extract, and Preparation of a Nanoemulsion by a Low-energy/Solvent-free Method, *In-silico* Study of β-sitosterol in Ulcerative Colitis. International Journal of Drug Delivery Technology. 2024;14(1):265-273.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Jatropha curcas L., also known as barbados nut or purging nut tree, belongs to the Euphorbiaceae family and is a small shrub or tree. Its leaves hold significant promise as a source of essential oil,[1] which has exhibited remarkable antibacterial and antioxidant properties. As a result, essential oil is gaining attention as a natural preservative in the pharmaceutical and cosmetic industries.[2] Notably, the plant contains various beneficial compounds, including terpenes, diterpenoids, phenolics, flavonoids, phytosterols, and saponins, which possess antioxidant, anticancer, anti-inflammatory, antimetastatic, coagulant, and anti-coagulant (dose-dependent), disinfectant, antiparasitic, wound healing, insecticidal, pregnancy termination, and antidiarrheal properties. Some of the key constituents found in the leaves include curcin, curcusone-B, curcain, β-sitosterol, and stigmasterol.

The pathogenesis of ulcerative colitis, a condition characterized by inflammation in the colon, remains unclear. Conventional therapy is currently the preferred treatment for this condition. Although there have been limited studies on herbal medicines, they show promising potential in managing ulcerative colitis. These days, stress is just part of being human. Inflammatory bowel disease (IBD) and other physical health problems may worsen with prolonged stress. Very little is known about the precise mechanisms behind this link. Chronic stress disrupts gut microbiota, which in turn triggers immune system responses and exacerbates dextran sulfate sodium-induced colitis, according to studies. It is thought that an uncontrolled immune response in the inflamed intestinal mucosa is what speeds up the development of IBD when TLR4/NF-B is overactivated.[5]

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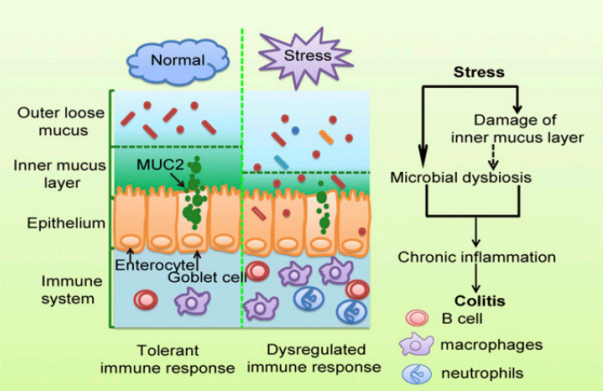

Figure 1: Pathogenesis of ulcerative colitis[5]

The phosphatidylinositol 3-kinase-protein kinase B pathway (PI3K-AKT) is a signaling network that plays a role in metabolism, cell survival, proliferation, and development, among many other biological processes. It regulates various biological functions and is typically dysregulated in diseases such as cancer and inflammatory disorders such as ulcerative colitis (UC).[6] In ulcerative colitis, continuous inflammation and immune dysregulation exist in the colon and rectum. "Several studies have been carried out to investigate the involvement of the PI3K-AKT pathway in the pathophysiology of UC. Growth hormones and cytokines, for example, can activate the PI3K-AKT pathway, resulting in downstream signaling events. TNF-alpha and IL-6, two pro-inflammatory cytokines, have been found in UC to activate the PI3K-AKT pathway in colonic epithelium and immune cells.[7] The PI3K-AKT pathway can be dysregulated, resulting in increased permeability and the transfer of hazardous chemicals across the gut lining. In UC, the PI3K-AKT pathway has emerged as a possible therapeutic target as shown in Figure 1."

Plant sterol-sitosterol is found in a range of fruits, vegetables, nuts, and seeds. It has a similar structure to cholesterol and is well-known for its potential health benefits. While -sitosterol has been studied for its potential advantages in a range of health conditions, including cardiovascular and prostate health, its role in the treatment of UC remains unknown. Some study, however, shows that -sitosterol may have anti-inflammatory properties and may help persons with UC.[6,8]

MATERIALS AND METHODS

RAW 264.7 Cell Line Studies[9]

The RAW 264.7 (NCCS) murine macrophage cell line was grown in DMEM at a glucose concentration of 1-g/L, with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate added. The cells were kept in a 37°C environment with a volume ratio of 5% CO_2 to 95% air. A density of 5×105 cells per mL of fluid was used to seed macrophage RAW 264.7 cells onto 24-well plates. Fresh DMEM with 0.5% FBS was added to the medium after 24 hoursof seeding. Following this, the cells were subjected to varying concentrations of the four selected compounds (3.125, 6.25, 12.5, 25, 50, & 100 M). To activate the cells, 1-µg/mL of LPS was administered to them after 2 hours. Finally, the supernatants were collected for ELISA analysis 24 hours after stimulation, as per the manufacturer's instructions. The purpose was to analyze the secretion of IL-6. The method used was a Mouse IL6 ELISA kit (ab2225030).

In-vivo Study-DSS Induced Colitis and Stress-induced Colitis[10]

- Mice utilized were 8-week-old BALB/C rats obtained from the Bangalore animal facility of Biogen. An Institutional Animal Ethics Committee at SRMIST's College of Pharmacy gave its stamp of approval to the study's procedures. Reference: IAEC/292/2022. A 10-day quarantine period was instituted for the animal as shown in the Table 1.
- Group 1-control;
- Group 2- standard-Prednisolone
- Group 3-2.5% DSS in drinking water for 7 days
- Group 4- Stress by water immersion at 6 hours /week
- Group 5+Group 6- DSS induced+ 100 mg/kg of test drug and 200 mg/kg of test drug
- Group 7+ Group 8 -DSS induced+ stress induced + 100 mg/kg of test drug and 200 mg/kg of test drug
- 8th Day sacrifice the animals
- Biochemical evaluation and histopathological studies.

Disease activity index[11]

The disease activity index (DAI) is a commonly used scoring system to assess disease severity and activity in experimental models of colitis. DSS-induced colitis is a widely used model to mimic certain aspects of human inflammatory bowel disease, such as ulcerative colitis. The DAI scoring system combines several parameters to evaluate the severity of colitis in mice over a specific period. The specific parameters and scoring criteria may vary slightly between studies, but here is a general outline of the parameters commonly included in the DAI for DSS-induced colitis:

- *Weight loss*

Percentage of body weight loss compared to the initial weight.

- *Score*

Typically graded on a scale of 0–4, with 0 indicating no weight loss and 4 indicating severe weight loss (>20%)."

Stool consistency

Assessment of fecal consistency or diarrhea.

- *Score*

Generally graded on a scale of 0–4, with 0 representing normal stool consistency and 4 indicating severe diarrhea.

Rectal bleeding

Evaluation of the presence and severity of rectal bleeding or haematochezia.

Score

Often graded on a scale of 0–4, with 0 indicating no bleeding and 4 representing gross bleeding.

Table 1: Animal grouping

S. No	*Animal*	*No of groups*	*No of animals*	*Dose*	*Route*
1	BALB/C	Control	6	Vehicle	Oral
2		Standard	6	Prednisolone (100 mg/kg)	ip
3		DSS induced	6	2.5% DSS in drinking water	Oral
4		Stress induced	6	Water immersion for 6 hours per week	-
5		DSS induced+ low dose	6	Low dose (100 mg/Kg)	Oral
6		DSS induced+ high dose	6	High dose (200 mg/Kg)	Oral
7		DSS induced+ Stress induced+ low dose	6	Low dose (100 mg/Kg)	Oral
8		DSS induced+ Stress induced+ high dose	6	High dose (200 mg/Kg)	Oral

General activity and appearance

Assessment of the mice's overall well-being, activity level, and appearance.

- *Score*

Normal look and activity level are denoted by 0, whereas excessive inactivity, slumped posture, or piloerection are indicated by 4.

Weight of the animal and size of the colon[11]

Observe the animals in each group and take note of the weight variance in each group. For both the control and model groups of mice, enemata gave saline. Daily weight measurements were taken from the animals. Day 7 saw the administration of anesthesia, the cervical dislocation of the mice, and the rapid collection of colon samples. Next, the length of the colon was measured and recorded for use in further research.

Formulation of Nanoemulsion[12-15]

The nanoemulsion is made from the leaves of the little shrub or tree *J. curcas* L., which is sometimes called a barbados nut tree or purging nut tree. As shown in the Table 2. It is a member of the Euphorbiaceae family. As an essential oil[1] source, its leaves show great potential because of its outstanding antibacterial and antioxidant characteristics. Consequently, the cosmetics and pharmaceutical sectors are beginning to recognize essential oil for its potential as a natural preservative.[2] The plant is rich in beneficial compounds that have various effects on health. Some of these compounds include terpenes, diterpenoids, phenolics, flavonoids, phytosterols, and saponins. These compounds have antioxidant, anti-inflammatory, antimetastatic, coagulant, disinfectant, antiparasitic, wound healing, insecticidal, antidiarrheal, and pregnancy termination properties. Some of the key constituents found in the leaves include curcin, curcusone-B, curcain, β-sitosterol, and stigmasterol.

Table 2: Preparation of nanoemulsion

Batch No	*Oil (%)*	*S_{mix} (%)*	*Water (%)*
F1	10	10	80
F2	10	15	75
F3	10	20	70
F4	12.5	12.5	75
F5	12.5	18.75	68.5
F6	12.5	25	62.5
F7	15	15	70
F8	15	22.5	62.5
F9	15	30	55

The pathogenesis of ulcerative colitis, a condition characterized by inflammation in the colon, remains unclear. Conventional therapy is currently the preferred treatment for this condition. Although there have been limited studies on herbal medicines, they show promising potential in managing ulcerative colitis. Stress is ubiquitous in today's fast-paced, competitive environment, and research has linked long-term stress to negative health outcomes, such as the worsening of inflammatory bowel disease (IBD). We still don't have a complete picture of the processes behind this connection. According to one research, dextran sulfate sodium-induced colitis is worsened when prolonged stress disturbs the gut flora, which in turn triggers immune system responses. The overactivation of TLR4/NF-B is proposed as a mechanism that leads to an uncontrolled immune response in the inflamed intestinal mucosa, thus accelerating the progression of IBD.[5]

Physical Characterization of Nanoemulsion[16,17]

Droplet size, PDI analysis, and zeta potential determination

The modified NE systems' droplet size distributions and polydispersity indices (PDIs) were examined three times using a Horiba Particle Size Analyzer that relies on dynamic light scattering. Prior to analysis, distilled water was used to dilute each sample at a ratio of 1:100. Similarly, the improved NE systems' zeta potentials were found by means of laser Doppler anemometry using a Zetasizer (Horiba). In order to conduct this analysis, 100 µL of each sample was additionally mixed with 1:100 distilled water. the results were shown in the Figures 2-7.

pH and viscosity determination

An electronic pH meter was used to ascertain the nanoemulsion's pH measurement. Two grams of nanoemulsion were balanced and mixed with 20 mLs of distilled water. All of the measures were taken three times, and the average was reached.

A Brookfield viscometer was employed to measure the viscosities of the nanoemulsion samples. The viscometer was equipped with a number 2 spindle immersed in the

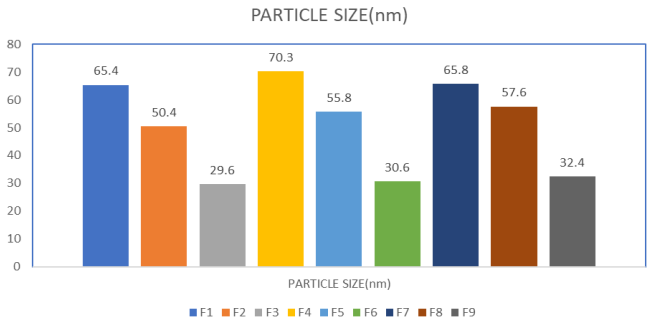

Figure 2: Particle size

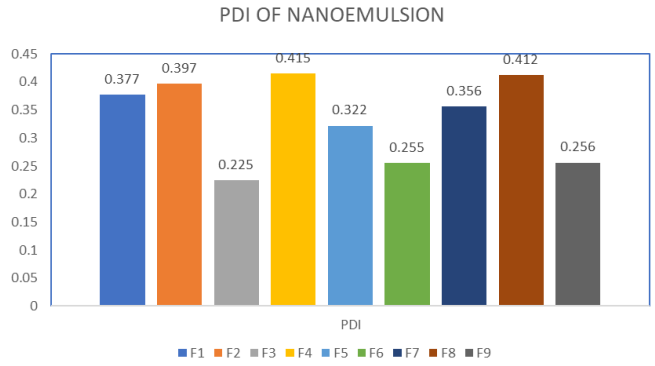

Figure 3: PDI

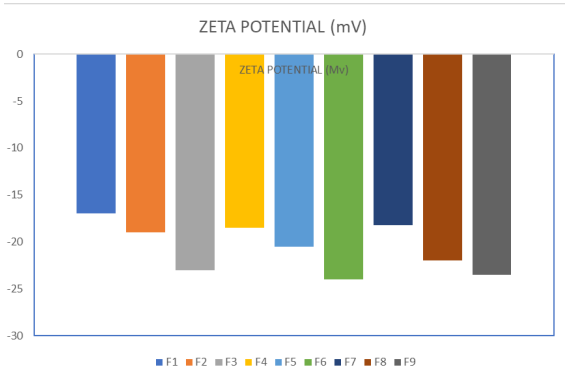

Figure 4: Zeta potential

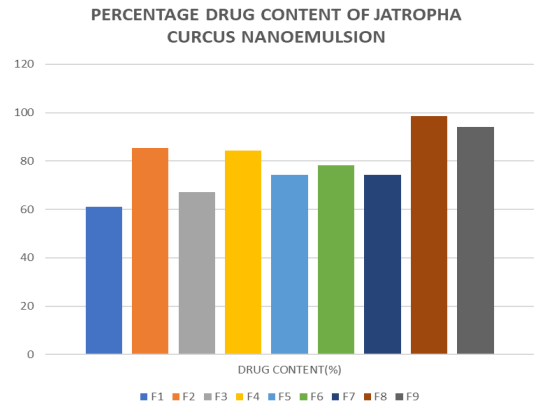

Figure 5: % Drug content of *J. curcus* nanoemulsion

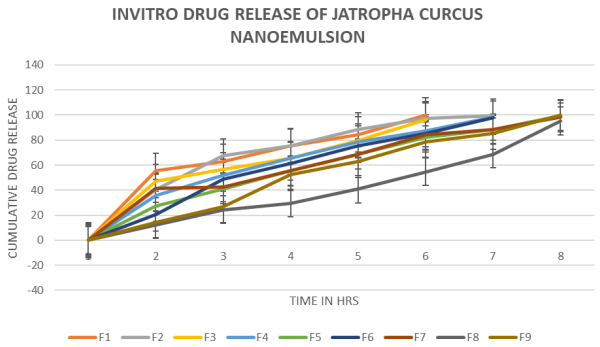

Figure 6: *In-vitro* drug release study

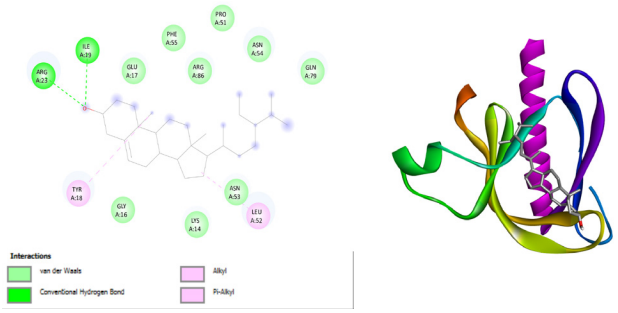

Figure 7: Docking score and 2D structure

Disease Activity Index

In the DSS-induced colitis model, the values for each parameter are added together to provide a cumulative disease activity index (DAI) score, which offers an overall evaluation of disease severity as shown in the Tables 5-10. Higher scores imply that the illness is progressing more rapidly. The DAI score is a composite score that considers numerous factors such as stool consistency, rectal bleeding, and weight loss. In comparison to the normal and drug-treated groups, animals in the DSS and stress-induced groups scored 4 on stool consistency. Some of the animals in group 5 had blood in their stools. In groups 6 and 8, there is no stool bleeding.

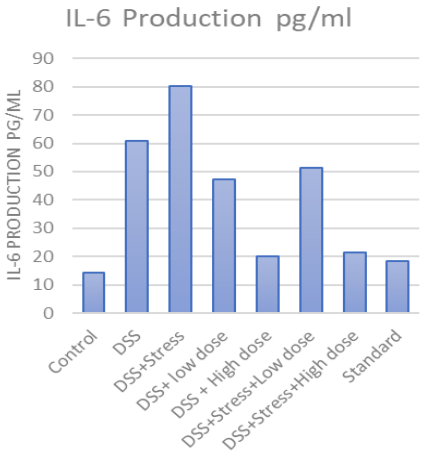

Figure 8: RAW 264.7 cell lines

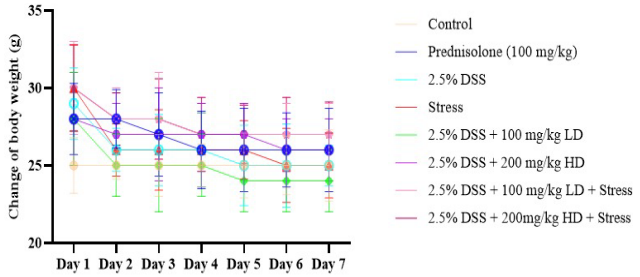

Figure 9: Size of the colon

Table 3: Evaluation of nanoemulsion

Formulation	*Particle size (nm)*	*PDI*	*Zeta potential (MV)*
F1	65.4	0.377	-17
F2	50.4	0.397	-19
F3	29.6	0.225	-23
F4	70.3	0.415	-18.5
F5	55.8	0.322	-20.5
F6	30.6	0.255	-24
F7	65.8	0.356	-18.2
F8	57.6	0.412	-22
F9	32.4	0.256	-23.5

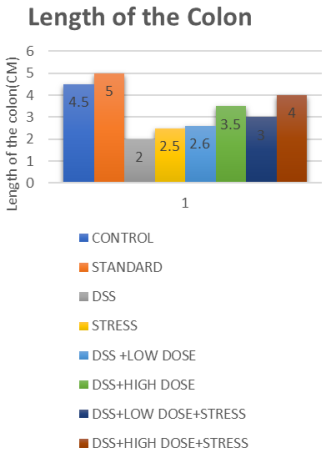

Figure 10: Length of the colon

nanoemulsion. The rotations were carried out at speeds of 5, 10, 20, 50, and 100 rpm at room temperature. At each speed, the readings were recorded on the viscometer. The results of pharmaceutical factors is shown in the Tables 3 and 4.

Thermodynamic stability study[17]

To determine the physical stability of the *Jatropha curcus* nanoemulsions (NEs), a centrifugation test was performed. During this stress test, we monitored for a variety of potential physical instabilities, including phase separations, drug precipitations, and color changes. Additional research and characterization of NEs did not include those displaying these instabilities. In Mumbai, India, a REMI International centrifuge was used to spin the prepared NEs at 5000 rpm for half an hour. We looked for indications of phase separation, creaming, or cracking in the samples after centrifugation.

Molecular Docking[18-19]

Molecular docking methods are usually the go-to when trying to figure out how ligands and proteins interact. The most popular docking method involves keeping the protein target stiff while allowing the ligand to explore flexible conformations; this approach is known as rigid-flexible docking.

Docking tests were conducted for the optimized compounds in this study using the Auto Dock 1.5.7 automated docking tool, which uses the Lamarckian Genetic Algorithm 19. An in-depth familiarity with the particular target-bioactive agent interactions is crucial for the development of novel pharmaceuticals. Auto Dock does this by merging two approaches: a powerful search for torsional flexibility and a quick estimate of energy based on the grid.

After obtaining the final binding energy values, the binding interactions were examined using the right techniques to learn about the ligand-protein binding interactions.

Pharmacokinetic Property[21]

The structure of the compound is acquired from the PubChem website. SWISS ADME online property prediction was used to determine physiochemical characteristics such as absorption, distribution, metabolism, and elimination of the specified chemical ingredients.

RESULTS

RAW 264.7 Cell Lines Study

The RAW 264.7 cell line is a popular choice among researchers in biomedicine due to its ability to imitate the immune response and secrete cytokines that promote inflammation. Although it is not approved for use in treating ulcerative colitis, this cell line is often employed in *in-vitro* experiments to study how different chemicals affect inflammation and immune responses. One hallmark of inflammatory bowel disease (IBD) is an uncontrolled inflammatory process in the intestines, and IL-6 plays a role in this. For CD4+ T cells to survive in the ulcerative colitis inflammation site, the transcription factor STAT-3 must be activated by IL-6 by transmigrating.

Therapeutic target Inhibition of IL-6 has shown promise as a therapeutic approach for ulcerative colitis. IL-6 plays a crucial role in the pathogenesis and inflammation associated with ulcerative colitis. Targeting IL-6 signaling may offer potential therapeutic benefits for individuals with this condition. IL-6 is elevated in inflammatory bowel disease, including ulcerative colitis. An important part of the immune response and inflammation is the pro-inflammatory cytokine interleukin-6 (IL-6) as shown in Figure 8.

In-vivo Results

Weight change in animal and length of the colon

Weight loss, blood in the stool, elevated DAI score, and shorter colon length were seen in the DSS-induced mice as compared to the control and standard groups.

Depending on the research design, the length of the colon in mice with UC can be quantified in a variety of methods. Morphological measurements of the colon length in mice with DSS-induced colitis were taken. The colon length was one of the biological markers utilized to measure the degree of inflammation in the colons of rats induced with DSS.[26] The length of the colon was measured after the mice were killed. After 5 days of DSS treatment, the animals' colon lengths decreased by almost 30%, which was the most noticeable effect. Using a ruler, we determined the colon's length. In contrast to the control groups, those with DSS-induced colitis had shorter colons (Figures 9 and 10).

Table 4: Drug content

Formulation code	*Drug content (%)*
F1	61.2
F2	85.3
F3	67.3
F4	84.3
F5	74.3
F6	78.2
F7	74.2
F8	98.6
F9	94.2

Molecular Docking

To analyze the physicochemical properties and the compounds' drug-likeness, the structure of β-sitosterol was sketched in the molinspiration program. As shown in Figure 9. β-sitosterol has been declared to have passed the "Lipinski's role of five" because of their improved lipophilicity, presence of sufficient H-bond donor and acceptor sites, rotatable bonds with low number, and small surface area is polar. Docking with AUTODOCK 4.2 was performed on β- Sitosterol.P13K-ATK receptor PDB file 1H10. The binding score is -5.3 kj3, as well as a negative energy difference. When compared to the typical protein drug, the Auto dock result reveals that the ligands β-sitosterol have a lower binding energy, indicating a stronger affinity with the proteins.[22-24]

Pharmacokinetic Analysis Results[22]

The field that studies the absorption, distribution, metabolism, and excretion (ADME) of pharmaceuticals is known as pharmacokinetics. as shown in the Tables 6-11. anticipate ADME parameters, pharmacokinetic features, druglike nature, medicinal chemistry friendliness, and physiochemical descriptors with the help of Swiss ADME, a free online application. Moleinspiration is another tool that can be used to calculate drug likeness. Osiris can be used to quantify some toxicity risk parameters. The in-silico methods of Swiss ADME can be used to analyze pharmacokinetic studies and

Table 5: *In-vitro* drug release study

Time (hrs)	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*
0	0	0	0	0	0	0	0	0	0
0.5	55.5	40.3	47.2	35.6	27.1	20.5	41.5	12.3	14.5
1	62.6	67.5	56.6	52	41	48.6	42.3	24.3	26.65
2	75.2	75.4	65.4	65.3	55.3	61.3	55.3	29.6	52.36
4	84.3	88.3	79.6	78.3	68.7	75.6	68.4	40.65	62.76
6	99.8	97.2	96.2	87.6	82.3	85.4	84.1	54.63	78.77
12		99.4		98.6	88.7	97.9	88.6	68.78	85.18
					99.6		98.2	95.22	99.7

Table 6: Pharmacokinetic analysis results (25)

Parameter	*β-sitosterol predicted value*
Water solubility (log mol/L)	-7.90
Intestinal absorption (human) (%Absorbed)	Low β-sitosterol
P-glycoprotein I/II inhibitor	No
VDss (human) (log L/kg)	-1.621
Fraction unbound (human) (Fu)	0.825
BBB permeability (log BB)	-0.082
CNS permeability (log PS)	-1.243
CYP2D6 substrate	No
CYP3A4 substrate	Yes
CYP1A2/2C19/2C9/ 2D6/3A4 inhibitor	No
Total clearance (log mL/min/kg)	-0.034
Max. tolerated dose (human) (log mg/kg/day)	0.025
hERG I/II inhibitor	No

Table 7: Physicochemical properties of β-sitosterol using Swiss ADME (24)

Parameter	*B-sitosterol predicted value*
Molecular weight	414.71 g/mol
Fraction Csp3	0.93
Num. heavy atoms	30
Num. arom. heavy atoms	0
Num. rotatable bonds	6
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar refractivity	133.23
TPSA	20.23 Å2

Table 8: Lipophilicity profile of β-sitosterol profile using Swiss ADME (24)

Parameter	*β-sitosterol value*
Log Po/w (iLOGP)	4.79
Log Po/w (XLOGP3)	9.34
Log Po/w (WLOGP)	8.02
Log Po/w (MLOGP)	6.73
Log Po/w (SILICOS-IT)	7.04
Consensus Log Po/w 6.97 7.19	

show Lipinski's rules. The ADMET lab web server can be used to predict physiochemical properties such as logS, logD, logP, absorption, distribution, metabolism, and excretion.

CONCLUSION

In this research, we investigated the therapeutic potential of *J. curcas* leaves extract, its nanoemulsion formulation, and

Table 9: Water solubility profile of β-sitosterol using Swiss ADME

Parameter	*β-sitosterol value*
Log S (ESOL)	-7.9
Solubility	5.23E-06 mg/mL;1.26E-08 mol/l
Class	Poorly soluble
Log S (Ali)	-9.67
Solubility	8.90E-08 mg/mL;2.15E-10 mol/l
Class	Poorly soluble
Log S (SILICOS-IT)	-6.19
Solubility	2.69E-04 mg/mL;6.49E-07 mol/l
Class	Poorly soluble

Table 10: Bioactivity score of β-sitosterol using molinspiration®

Parameter	*β-sitosterol value*
GPCR ligand	0.14
Ion channel modulator	0.04
Kinase inhibitor	-0.51
Nuclear receptor	0.73
Protease inhibitor	0.07
Enzyme inhibitor	0.51

Table 11: Toxicity profile of β-Sitosterol using OSIRIS property explorer

Parameters	*β-sitosterol scores*
Mutagenic	Green
Tumorigenic	Green
Irritant	Green
Reproductive effect	Green
TPSA	20.23
Drug likeness	1
Drug Score	0.4"

the role of β-sitosterol in UC. The findings from our *in-vitro*, *in-vivo*, and *in-silico* investigations collectively shed light on the efficacy and underlying mechanisms of *J. curcas* as a potential alternative treatment for UC.

Interleukin-6 (IL-6) is one of many inflammatory mediators and cytokines produced by activated RAW 264.7 cells with phagocytic activity. Lipopolysaccharide (LPS) is one of the substances that may activate RAW 264.7 cells to launch an inflammatory response. When exposed to LPS, cells begin to produce inflammatory cytokines *via* the activation of the Toll-like receptor 4 (TLR4) signaling pathway. *In-vitro* studies on RAW 264.7 cell lines demonstrate an anti-ulcerative colitis effect due to a decrease in IL-6 production. *J. curcas* leaf extract has the ability to reduce oxidative stress and the inflammatory response linked to ulcerative colitis, according

to *in-vitro* research conducted using RAW 264.7 cells, which also shown strong antioxidant and anti-inflammatory effects.

The *in-vivo* study supported the *in-vitro* findings, as the administration of *J. curcas* leaf extract to the DSS-induced model resulted in a dose-dependent reduction in disease severity. The extract demonstrated its ability to ameliorate colon tissue damage, reduce weight variation and reduce pro-inflammatory cytokine levels, suggesting its potential to modulate the inflammatory process in ulcerative colitis.

The preparation of a nanoemulsion by a low-energy and solvent-free method offered an innovative drug delivery approach to enhance the bioavailability and efficacy of the *J. curcas* leaves extract. The nanoemulsion exhibited improved stability, increased encapsulation efficiency, and sustained release of active components, making it a promising platform for targeted delivery and controlled release of therapeutic agents in UC treatment.

The *in-silico* investigation exploring the molecular interactions of β-sitosterol with key inflammatory targets provided valuable insights into the mechanism underlying its anti-inflammatory effects. Strong binding affinities between β-sitosterol and inflammatory mediators in the P13K-AKT pathway suggested its potential role in regulating the inflammatory cascade in UC.

Overall, the findings of this interdisciplinary investigation suggest the potential of *J. curcas* leaves extract and its nanoemulsion formulation as prospective UC therapy options. The combination of *in-vitro*, *in-vivo*, and *in-silico* techniques aided in gaining a thorough knowledge of their therapeutic effects and mechanisms of action. SWISS ADME provides useful information about drug similarity, physicochemical qualities, drug metabolism, and other ADME-related factors. Some examples are lipophilicity, solubility, absorption, permeability, metabolism, protein binding, and toxicity. The tool employs various techniques and models to estimate these qualities. Examine the expected pharmacokinetic parameters and explain their significance. Lipophilicity (reported as logP or logD) predicts a compound's capacity to penetrate cell membranes, whereas solubility predicts the compound's ability to dissolve in aqueous solutions.

More study is necessary to enhance the nanoemulsion's formulation for clinical use and to confirm these results in clinical trials. Furthermore, more focused treatments for UC might be developed by clarifying the molecular mechanisms by which β-sitosterol achieves anti-inflammatory actions.

Finally, the findings of this study lay a strong platform for the future development of *J. curcas* -based medicines and nanoemulsion delivery systems as prospective therapeutic approaches for ulcerative colitis.

ACKNOWLEDGEMENT

We are grateful to the heads of the Department of Pharmacology for their unlimited cooperation and support in data collection process. Special thanks to my guide for the continous moral support and encouragement.

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

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