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# FORMULATION AND PRECLINICAL EVALUATION OF NIOSOMES CO-LOADED WITH 5-FLUOROURACIL AND LEUCOVORIN

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## ABSTRACT

In the present study, niosomes co-loaded with 5-Fluorouracil and Leucovorin was prepared and evaluated for *in vivo* anticancer efficacy in Dimethyl hydrazine (DMH) induced colon cancer. In the present study, colon cancer was induced by s.c injection of DMH (20 mg/kg b.wt) for 15 weeks. The animals were divided into five groups as follows control, DMH alone, DMH and 5-FU, DMH and 5-FU + LV market formulation and DMH and 5-FU + LV niosomes formulation and the treatment was carried out for 15 weeks. At the end of the study period the blood was withdrawn and serum was separated for haematological, biochemical analysis and tumor markers. Further, the colonic tissue was removed for the estimation of antioxidants and histopathological analysis. The results of the study displays that DMH intoxication elicits altered haematological parameters (RBC, WBC and Hb), elevated lipid peroxidation and decreased antioxidants level (SOD, CAT, GPX, GST and GSH), elevated lipid profiles (cholesterol and triglycerides), tumor markers (CEA and AFP) and altered colonic tissue histology. Mean while, treatment with 5-FU + LV niosomes significantly restored the altered biochemicals parameters in DMH induced colon cancer mediated by its anticancer efficacy. Further, 5-FU + LV niosomes showed marked efficacy as that of the 5-FU + LV market formulation and 5-FU-alone.

## KEYWORDS

Niosomes, Multiple drug loading, Leucovorin, 5-Flurouracil, Colon cancer, Dimethyl hydrazine, Antioxidant and Lipid peroxidation.

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## INTRODUCTION

In recent years, niosomes as a drug carrier has been received much attention in pharmaceutical academia and industrial research. Generally niosomes have a bilayer structure and are formed by self-association of nonionic surfactants and cholesterol in an aqueous phase[1]. It has many advantages like biodegradable, biocompatible, and nonimmunogenic, long shelf life, exhibit high

stability and achieves the delivery of drug at target site in a controlled and/or sustained manner. Also niosomes may alleviate the disadvantages associated with liposomes such as chemical instability, variable purity of phospholipids and high cost[2]. Encapsulation of a large number of drugs with a wide range of solubility in niosomes using various types of nonionic surfactants has been extensively studied[3].

Combination therapy with drugs of different therapeutic effects shows an improving efficacy in the treatment of various diseases. Particularly, cancer treatment requires simultaneous administration of different combination of drugs due to the molecular complexity of cancer diseases[4,5]. Niosomes show potential in combination of drug delivery and targeting combine transdermal and tumor targeting ability in cancer therapy[6,7]. 5-Fluorouracil (5-FU) has been in clinical use as an anticancer drug for more than 30 years. Although 5-FUhas a broad spectrum of anticancer activity including common solid tumors present in the gastrointestinal system. But only a minority of patients treated with 5-FU experience an objective response to therapy[8]. Leucovorin is an active metabolite of folic acid and an essential coenzyme for nucleic acid synthesis. Leucovorin may significantly increase both the clinical efficacy and the clinical toxicity of 5-FU in cancer patients. From literature we found that 5-Flurouracil in combination with leucovorin is well acknowledged in the treatment of cancer[9-11]. But only a few approaches consider the developing new drug delivery systems co-loaded with 5-FU and Leucovorin has been investigated.

The purpose of this study was to prepare 5-FU and Leucovorin niosomes formulation and evaluate its anticancer efficacy in Dimethyl hydrazine (DMH) induced colon cancer in a animal model.

## MATERIAL AND METHODS

### Drugs and Chemicals

5-Fluorouracil and Leucovorin was procured from Sigma Aldrich, India. Cholesterol, polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60) and dicetyl phosphate (DCP) were procured from Merck, India. All materials used in the study were of analytical grade.

### Preparation of 5-FU and Leucovorin Niosomes

Niosomes were prepared by a thin film hydration technique using a mixture of surfactants encompassing (span 40, span 60, tween 60 and tween 40) and cholesterol, at different specified ratios as given in Table No.1. Surfactant and cholesterol was dissolved in 8 ml of diethyl ether and the drugs were dissolved in 2 ml of ethanol. The mixture was then transferred to a round bottom flask, and the solvent was evaporated under reduced pressure at a temperature 20-25º, using a rotary flash evaporator until the formation of a thin lipid film. The resultant film was made wet with 10 ml of phosphate buffer saline pH 7.4. The hydration was continued for 1 h, while the flask was kept rotating at 55-65°. The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain niosomal dispersion.

### Evaluation of 5-FU and Leucovorin Niosomes

The evaluation of niosomes was carried in our laboratory as described in the previously published paper[12].

### Preclinical anti colon cancer evaluation of 5-FU and Leucovorin Niosomes

### Chemicals

The 1, 2-dimethyl hydrazine (DMH) was obtained from Sigma Aldrich, India and all other chemicals and reagents used were of analytical grade.

### Animals

Male Sprague-Dawley rats (300-400gm body weight) were procured from KMCH college of Pharmacy, Coimbatore, Tamilnadu, India. All the animals were kept at room temperature of 22°C under 12 hr light/12 hr dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. All animal procedures were performed in accordance with the recommendation of CPCSEA the proper care and use of laboratory animals the proposal of the present study was approved by IAEC of KMCH college of Pharmacy, Coimbatore, Tamilnadu, India.

**Preparation of DMH solution**

DMH was dissolved in 1 mm EDTA just prior to use and the pH was adjusted to 6.5 with 1mm sodium bicarbonate to ensure the stability of the chemical.

**Induction of colon cancer**

Animals were given a weekly subcutaneous (s.c.) injection of DMH in the groin area at a dose of 20 mg/kg body weight for 15 weeks.

**Study design**

The animals were divided into five groups of six animals each as follows,

Group I - Control, received 1 ml of Normal saline. p.o everyday for 15weeks.

Group II - Received DMH (20mg/kg body weight once in a week for 15 weeks, s.c)

Group III - Received DMH (20mg/kg body weight once in a week for 15 weeks, s.c) +5 fluorouracil (20mg/kg) i.p.

Group IV - Received DMH (20mg/kg body weight once in a week for 15 weeks, s.c) +5-FU and Leucovorin market formulation.

Group V - Received DMH (20mg/kg body weight once in a week for 15 weeks, s.c) +5-FU and Leucovorin Niosomes formulation.

**Blood Collection**

After end of treatment period, the animals were anaesthetized with ketamine 2mg/kg (i.proute), blood was collected by Retro orbital puncture, with EDTA and 7 without EDTA for the enumeration of blood cell (i.e. RBC, WBC,) estimation of Hemoglobin and for estimation of various biochemical parameters respectively.

**Separation of Serum**

For estimating the biochemical parameters such as SGOT, SGPT, ALP and total protein the serum was separated from blood by centrifuging at 10,000 rpm for 10 minutes. The separated serum were collected and used for the parameter estimation.

**Separation of Plasma**

For the estimation of tumour markers such as Alpha – feto - protein (AFP), Carcinoembroyonicantigen (CEA), the blood was collected with EDTA, and centrifuged at 10,000 rpm for 5 min and the separated plasma was used for the parameter estimation

**Estimation of Haematological parameters**

Total Leukocyte count (TLC), total erythrocyte count (TEC) and hemoglobin (Hb) content were determined by auto analyzer according to methods described by Dacie and Lewis[13].

**Assay of serum hepatic markers**

The level of serum glutamate oxalo acetate transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) was determined by the method of Reitman and Frankael[14]. The level of alkaline phosphatase (ALP) was estimated by the method of Plummer[15] and the protein was measured by the Lowry et al method[16].

**Estimation of Lipid profiles**

The extraction of serum lipids was done according to the procedure of Folch *et al*[17]. The estimation of total cholesterol was carried out by the method of Zlatkis *et al*[18] and triglycerides by the method of Foster and Dunn[19].

**Analysis of Serum tumor markers**

The serum hepatic tumor markers - α-feto protein (AFP) and carcinoembryonic antigen (CEA) were measured using ELISA assay kits from USCN LIFE science and technology (Wuhan, China).

**Estimation of oxidative stress parameters**

The colonic tissue level of superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich[20]. Catalase (CAT) level was estimated by the method described by Sinha[21]. Glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al*[22]. Glutathione S-transferase (GST) was assayed by the method of Habig *et al*[23]. Reduced glutathione (GSH) was determined by the method of Ellman[24].

**Estimation of Lipid peroxidation:**

The colonic tissue lipid peroxide level was determined as MDA by the method of Ohkawa *et al*[25]. The absorbance was measured photometrically at 532 nm and the concentrations were expressed as nmol malondialdehyde (MDA) min/mg/protein.

**Histopathology Studies**

The colon was fixed for 24 h in 10 % buffered formalin solution for histological study. Then a 2 × 2 mm dissected tissue was then sectioned (5 μm thickness), embedded and stained with haematoxylin and eosin (H and E).

**Statistical Analysis**

Values were expressed as mean ± SEM. The variation between groups was estimated by the one-way ANOVA followed by Dunnet's multiple comparison test using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA).

## RESULTS AND DISCUSSION

In our study, the formulation F4of 5-FU and Leucovorin noisome was found to satisfactory based upon the various parameters done for the evaluation of niosomes[12].

Colon carcinogenesis is a multistage process which involves initiation, promotion, and progression phases. Thus, the multistage sequence of events has many phases for prevention and intervention. Long term inflammation, is a hallmark risk factor for the progression of development of cancer in humans. DMH is a chemical carcinogen known to cause colon cancer with a reproducible preclinical *in vivo* system for evaluating "sporadic" (nonfamilial) forms of colon carcinoma[26]. Metabolism of DMH leads to the formation of AOM, MAM, and methyl diazonium ions which involves colonic mucosal DNA alkylation process. The metabolite, AOM, is a cardinal moiety involved in the methylation at the O-6 position of guanine, occurs within 6 to 12 h of DMH injection.

In our study, haematological parameters were significantly altered in the DMH treated as a carcinogenesis and altered immune function. The level of RBC and haemoglobin were significantly ($p<0.05$) reduced in DMH groups, whilst the WBC level was significantly ($p<0.05$) increased as that of the control rats. Meanwhile, treatment with 5-FU+ LV niosomes significantly ($p<0.05$) restored the altered blood parameters level to normalcy (Table No.2). The present result show that, the colonic tissue level of the LPO (Table No.3) increased significantly ($p<0.05$) and GSH (Table No.2), SOD, CAT, GPx and GST level (Table No.4) were decreased significantly ($p<0.05$) in DMH alone group compared with normal control group. Suggest that rapid cell proliferation involved in colon cell. Hence, cancer cell have certain characteristics that promote proliferation and tend to faster cell proliferation[27]. DMH itself can generate $H_2O_2$ in the presence of copper ions. In the presence of metal ions such as $Fe^{2+}$ and $Cu^{2+}$, $H_2O_2$ can react with $O_2$ to convert it into the more reactive OH radical. If sufficient amounts of CAT or GPx are not available to decompose $H_2O_2$, the generated OH radicals are capable of attacking DNA basement. GSH an important non-protein thiol in conjunction with GPx and GST plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging reactive oxygen species[28]. The present study, correlates with the decline in circulatory antioxidants such as SOD, CAT, GPx and GSH, and. This may be due to their accelerated synthesis to inhibit the lipid peroxidation end products the products of lipid peroxidation as well as sequestration by tumor cells. GPx uses $H_2O_2$ to catalyse the oxidation of GSH to GSSG, thereby nullifying the deleterious effects of $H_2O_2$[29]. Diminished GPx activity indicates cellular accumulation of the lipid hydro peroxides, which can potentially turn on a chain reaction, wherein more polyunsaturated fatty acid become targets for further per oxidative tissue injury. In the present study, GPx level was reduced in colonic tissue, which could be due to the elevated levels of hydro peroxides. Whilst, treatment with 5-FU+ LV niosomes significantly ($p<0.05$) decreased the level of MDA in colonic tissue of DMH treated rats, as well as significantly ($p<0.05$) increased the level of the antioxidants defense enzymatic system of SOD, CAT, GPx and GSH level.

Previous studies elicits that xenobiotic like DMH causes an increase in nonspecific microsomal enzyme activities of hepatic microsomal HMG -Co A reductase which mediates the cholesterol synthesis process. DMH also provoke hepatic damage and consequently results in loss of feedback control of cholesterol synthesis in liver, thereby elevating the level of bile acids and cholesterol in the bowel and serum[30,31]. In our study, serum level of cholesterol and triglycerides were significantly ($p<0.05$) elevated in DMH induced group may be due to higher cholesterogenes is induced by DMH. Whilst, treatment with 5-FU+ LV niosomes in DMH induced rats significantly ($p<0.05$) reduced

the serum level of total cholesterol and triglycerides (Table No.5).

The hepatic cell membrane damage releases the enzymes SGOT, SGPT, ALP into circulation, which can be measured in serum. High levels of SGOT indicate liver damage. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury. Previous studies indicate the hepatotoxicity role of DMH during the event of colon cancer[32]. In our study, DMH intoxicated rats displayed significantly ($p<0.05$) elevated level of hepatic marker enzymes (SGOT, SGPT and ALP) in serum and treatment with 5-FU+ LV niosomes significantly ($p<0.05$) decreased the hepatic markers enzyme level and restores the hepato cellular membrane damage elicited by DMH (Table No.6).

CEA is the best marker in colorectal cancer patients and also most thoroughly characterized tumour-associated antigens, in both biochemical and clinical aspects[33]. AFP is rarely reported until now in colorectal cancer compared to other cancers but it can also be an indicative of colorectal cancer[34]. In our study, serum level of CEA and AFP were significantly ($p<0.05$) elevated and 5-FU+ LV niosomes treatment significantly ($p<0.05$) reduced the level of tumor markers (CEA and AFP) to normalcy (Table No.7).

Further, the histopathological analysis revealed the thick enedmucosa with densely packed inflammatory cell in filtration and a higher degree of hyperplasia in DMH intoxicated rats and treatment with 5-FU+ LV niosomes showed normal appearing glands with normal mucosa of colon (Figure No.1).

**Units**

**RBC**

Millions /µl of blood; WBC: thousands/µl of blood; Hb: g/dl. The values are expressed as mean ±S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test. The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU +LV) Niosomes* denotes statistically significant $p< 0.05$.

**GSH**

Reduced Glutathione (nmole/mg of tissue); MDA: Malondialdehyde (nmole/mg of tissue). The values are expressed as mean ±S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test. The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU +LV) Niosomes* denotes statistically significant $p< 0.05$.

**SOD**

Superoxide dismutase (U/mg protein); CAT: Catalase (nmoles of H2O2 utilized/ min/ mg/ protein); GPx: Glutathione peroxidase (nmoles of GSH utilized/ min/ mg protein); GST: Glutathione - S- Transferase (nmoles of CDNB conjugate formed/ min/ mg/ protein). The values are expressed as mean ± S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test. The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU+LV) Niosomes. *denotes statistically significant $p< 0.05$.

The values are expressed as mean ±S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test. The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU +LV) Niosomes* denotes statistically significant $p< 0.05$.

The values are expressed as mean ±S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test. The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU +LV) Niosomes* denotes statistically significant $p< 0.05$.

**CEA**

Carcinoembryonic Antigen; AFP - Alpha – Feto - Protein. The values are expressed as mean ±S.E.M, n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test.

The comparison were made between a- Control vs DMH; b - DMH vs DMH+5-FU; c- DMH vs DMH + (5-FU +LV) Market formulation; d- DMH vs DMH + (5-FU +LV) Niosomes denotes statistically significant $p< 0.05$.

**Table No.1: Formulation composition of 5-FU and Leucovorin Niosomes**

| S.No | Type of formulation | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Drug | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | Cholesterol | 10 | 20 | 10 | 20 | 10 | 20 | 10 | 20 |
| 3 | Span 40 | 10 | 10 | - | - | - | - | - | - |
| 4 | Span 60 | - | - | 10 | 10 | - | - | - | - |
| 5 | Tween 40 | - | - | - | - | 10 | 10 | - | - |
| 6 | Tween 60 | - | - | - | - | - | - | 10 | 10 |
| 7 | Drug: cholesterol: SA | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 |

**Table No.2: Effect of 5-FU + LV niosomes on Haematological parameters on DMH induced colon**

| S.No | Groups | RBC | WBC | Hb |
|---|---|---|---|---|
| 1 | Control | 7.53±0.17 | 2.53±0.11 | 14.48±0.26 |
| 2 | DMH | 3.21±0.14$^{a*}$ | 8.42±0.16 $^{a*}$ | 8.97±0.16 $^{a*}$ |
| 3 | DMH+5-FU | 4.40±0.13$^{b*}$ | 5.64±0.17 $^{b*}$ | 11.23± 0.21 $^{b*}$ |
| 4 | DMH + (5-FU +LV) Market formulation | 5.87±0.16$^{c*}$ | 4.32±0.12 $^{c*}$ | 12.24±0.23 $^{c*}$ |
| 5 | DMH + (5-FU +LV) Niosomes | 6.40±0.18$^{d*}$ | 3.15±0.09 $^{d*}$ | 13.89±0.18 $^{d*}$ |

**Table No.3: Effect of 5-FU + LV niosomes on GSH and MDA level in DMH induced colon cancer**

| S.No | Groups | GSH | MDA |
|---|---|---|---|
| 1 | Control | 4.98±0.54 | 1.4±0.12 |
| 2 | DMH | 1.52±0.15 $^{a*}$ | 4.24±0.35 $^{a*}$ |
| 3 | DMH+5-FU | 2.67±0.21$^{b*}$ | 3.47±0.26 $^{b*}$ |
| 4 | DMH + (5-FU +LV) Market formulation | 3.12±0.29$^{c*}$ | 2.42±0.18 $^{c*}$ |
| 5 | DMH + (5-FU +LV) Niosomes | 3.92±0.42$^{d*}$ | 1.86±0.16 $^{d*}$ |

**Table No.4: Effect of 5-FU + LV niosomes on colon antioxidant enzymes in DMH induced**

| S.No | Groups | SOD | CAT | GPx | GST |
|---|---|---|---|---|---|
| 1 | Control | 7.84±0.75 | 7.14±0.68 | 2.34±0.05 | 0.75±0.006 |
| 2 | DMH | 2.89±0.29 $^{a*}$ | 2.63±0.25 $^{a*}$ | 0.57±0.01 $^{a*}$ | 0.25±0.003 $^{a*}$ |
| 3 | DMH+5-FU | 4.87±0.35 $^{b*}$ | 4.32±0.29 $^{b*}$ | 1.6±0.03 $^{b*}$ | 0.48±0.002 $^{b*}$ |
| 4 | DMH + (5-FU +LV) Market formulation | 5.92±0.37 $^{c*}$ | 5.75±0.42 $^{c*}$ | 1.98±0.03 $^{c*}$ | 0.65±0.005 $^{c*}$ |
| 5 | DMH + (5-FU +LV) Niosomes | 7.12±0.67 $^{d*}$ | 6.87±0.54 $^{d*}$ | 2.12±0.04 $^{d*}$ | 0.71±0.06 $^{d*}$ |

**Table No.5: Effect of 5-FU + LV niosomes on serum lipid profiles in DMH induced colon cancer**

| S.No | Groups | Total Cholesterol (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|
| 1 | Control | 76.94±2.46 | 122.6±3.33 |
| 2 | DMH | 171.4±7.65 [a*] | 164.4±2.68 [a*] |
| 3 | DMH+5-FU | 110.7±2.05 [b*] | 147.1±3.7 [b*] |
| 4 | DMH + (5-FU +LV) Market formulation | 90.44±2.47 [c*] | 138.28±1.88 [c*] |
| 5 | DMH + (5-FU +LV) Niosomes | 81.17±1.08[d*] | 125.5±5.23[d*] |

**Table No.6: Effect of 5-FU + LV niosomes on serum hepatic markers enzyme and total protein level in DMH induced colon cancer**

| S.No | Groups | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | Tot. Protein (mg/dl) |
|---|---|---|---|---|---|
| 1 | Control | 56.87±3.23 | 66.45±3.76 | 144.34±7.98 | 8.22±0.50 |
| 2 | DMH | 132.65±5.76 [a*] | 124.32±6.84 [a*] | 304.65±9.65[a*] | 5.21±0.31 [a*] |
| 3 | DMH+5-FU | 98.76±4.25 [b*] | 92.12±3.25 [b*] | 197.65±6.87[b*] | 6.76±0.35[b*] |
| 4 | DMH + (5-FU+LV) Market formulation | 86.87±5.65 [c*] | 79.67±4.82 [c*] | 175.42±4.56[c*] | 7.02±0.25[c*] |
| 5 | DMH + (5-FU +LV) Niosomes | 62.76±3.29 [d*] | 64.65±3.98 [d*] | 153.89±5.25[d*] | 8.02±0.50 [d*] |

**Table No.7: Effect of 5-FU + LV niosomes on tumour markers in DMH induced colon cancer**

| S.No | Groups | CEA (ng/dl) | AFP (ng/dl) |
|---|---|---|---|
| 1 | Control | 0.21±0.002 | 0.45±0.01 |
| 2 | DMH | 1.24±0.007 [a*] | 1.62±0.05 [a*] |
| 3 | DMH+5-FU | 0.56± 0.006 [b*] | 0.89±0.04 [b*] |
| 4 | DMH + (5-FU +LV) Market formulation | 0.45±0.003 [c*] | 0.72±0.03 [c*] |
| 5 | DMH + (5-FU +LV) Niosomes | 0.29±0.005 [d*] | 0.55±0.04 [d*] |

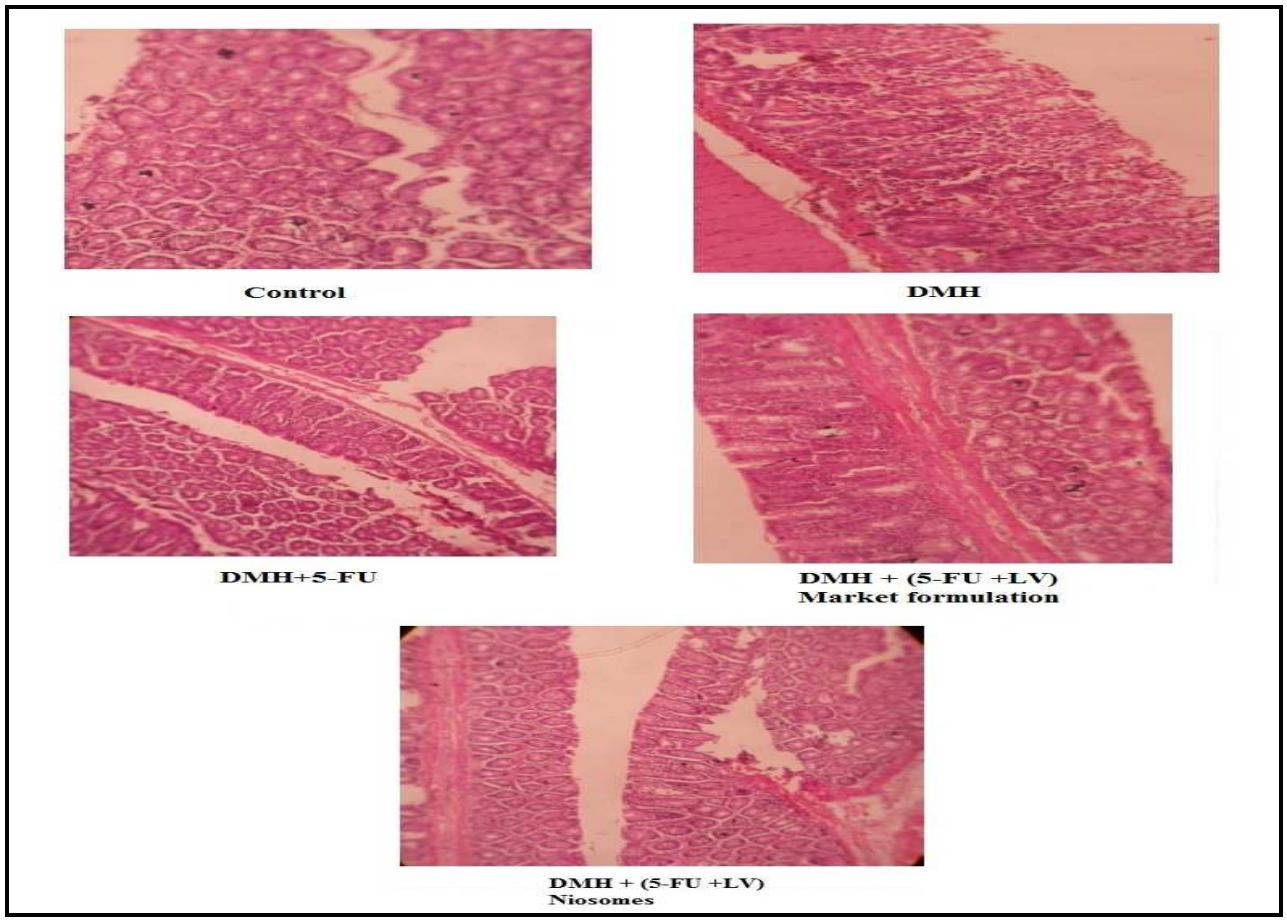


**Figure No.1: Effect of 5-FU + LV niosomes on colon histology in DMH induced colon cancer**

## CONCLUSION

Niosomal formulations containing 5- Fluorouracil and leucovorin were successfully prepared with different surfactants like Span 40, Span 60, Tween 40, and Tween 60 by thin film hydration technique. Further, preclinical evaluation of prepared 5-FU+ LV niosomes displayed efficient anticancer potential in DMH induced colon cancer by restoration of altered biochemical levels. Furthermore, the in our study 5-FU+ LV niosomes displayed marked anticancer efficacy as that of the 5-FU+ LV market formulation and 5-FU alone.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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