

IN VIVO EVALUATION OF NANOSTRUCTURED LIPID CARRIER SYSTEMS (NLCs) IN RATS BEARING BREAST TUMORS

Abstract

The focus of this study is the development of nanostructured lipid carriers (NLCs) as carriers for two anticancer drugs with remarkable antiproliferative potential. The maximal inhibition of cell growth by Raloxifene (RLX) & Curcumin (CUM) nanostructured lipid carriers (RLX-CUM-NLCs) was determined by assessing the viability of MDA-MB 231 cells. As far as we know, this is the first research to look at the effects of RLX-CUM-NLCs on DMBA-induced breast carcinogenesis in a rat model. RLX-CUM-NLCs reduced the number of tumors in an *in-vivo* investigation. After 14 weeks of induction, we discovered a tumour with a 100% incidence rate. The incidence of experimental breast cancer was decreased to 83.33% in the RLX-treated group. In contrast, RLX-CUM-NLCs demonstrated a significant anticancer effect with a 50% incidence in the RLX-CUM-NLCs group. Compared to controls, the RLX-CUM-NLCs therapy did not cause any toxicity in the animals in terms of food intake, body weight, or activity levels until 300 mg/kg BW. The current research shows that the RLX-CUM-NLCs has a chemopreventive impact on DMBA-induced breast cancer in rats by decreasing tumor burden and restoring marker enzymes activity.

Keywords: Nanostructured lipid carriers; Antineoplastic activity; Tumorigenesis; DMBA

INTRODUCTION

Breast cancer is the most often diagnosed cancer in women and the leading cause of death. Metastasis and tumor recurrence are posing new problems in the management of breast cancer [1]. Nowadays, breast cancer treatments comprise surgery, chemotherapy, radiation, hormone and immunotherapy; however, these therapies have side effects. Moreover, the 5-year disease-free survival rate of stage II breast cancer is 75–90%, ~30% for stage III patients and 0–10% for stage IV breast cancer [2], highlighting the need for new treatment and prevention strategies. Natural products are being used and investigated for the discovery and development of new therapeutic antineoplastic compounds [3]. Previously, several studies were done on CUM and RLX for its antitumorigenic action in a breast cancer promoting a significant reduction in tumor cells. In the study, development of nanostructured lipid carriers (NLC) as carriers for two antitumor compounds that possess a remarkable antineoplastic activity [4]. Recently, NLC has been described as a promising drug delivery system for enhanced cancer therapy [5]. Among the advantages of the NLC is the retention of the drug in the lipid core to promote the control of release. NLCs are second-generation Solid

lipid nanoparticles (SLNs) which are most effective drug delivery system for anticancer drug targeting [5]. Pure solid lipids are crystalline, and they prefer to stay in the β -polymorphic state, which is low in energy and well organized [5]. The lipid re-arranges into less stable forms when it is molten and cool during the synthesis of SLNs: polymorphic shape and form. These lipids are amorphous, which allows medicines to be retained inside the lipid matrix [6]. By replacing liquid lipid for a component of pure solid lipid, strategies to keep defects in the lipid, even after lengthy storage, alleviate the issues associated with standard SLNs [7]. When a little amount of liquid lipid/oil was added to a solid lipid matrix, the crystal lattice structures were less ordered [8]. Liquid lipids contributed in widening the loading capabilities of lipid nanocarriers by increasing the number of defects wherein amorphous drug clusters might fit [9]. As a consequence, this dual lipid framework may not only be able to accommodate higher drug load, but it may also be able to reduce drugs expulsion from lipid during storage [10].

Mingzhen Lin *et al.* [11] formulated a folic acid-loaded curcumin nanostructured lipid carrier using a solvent diffusion approach. The results revealed that FA-CUM-NLCs were efficient in selective delivery to cancer cells overexpressing FA receptors (FRs). CUM is also delivered to Breast cancer cells via FA-CUM-NLCs, boosting anti-tumor action. As a consequence, FA-CUM-NLCs might be a more effective nanomedicine for tumor therapy. The dual drug delivery offers several advantages such as dose reduction, overcoming of multidrug resistance and can result in synergistic effects [12]. In this paper we have discussed the in vitro cytotoxicity assay, in vivo study, acute toxicity study, Haematological and biochemical analyses and histopathological studies which shows that RLX-CUM-NLCs has a chemopreventive impact on DMBA-induced breast cancer in rats by decreasing tumor burden and restoring marker enzyme activity.

MATERIAL AND METHOD

Materials

Chemicals and Reagents

7, 12-Dimethylbenz(a)anthracene (DMBA) Cas No. 57-97-6 and other fine chemicals were purchased from Sigma Chemical Co. Raloxifene (RLX) was purchased from Anchrom Laboratories Mumbai. Curcumin was purchased from Amrita Herbal Sanwer road Indore MP. All other chemicals and solvents used were of analytical grade and highest purity.

Cells lines

The MCF-7 human breast cancer cell lines (MCF-7 cells) were obtained from the NCCS, Pune, India and cultured in Dulbecco's Modified Eagle's medium (DMEM)-High glucose media - (Cat No: AL149, Himedia). Both media were supplemented with 10% FBS and 1% antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin). The cells were maintained at 5% CO₂, 18-20% O₂ at 37⁰C temperature in the CO₂ incubator and subcultured every 2days.

Animals

Female rats of weighing 190±20 g was selected and procured from Animal house of PBRI. The animals were maintained under standard conditions of humidity, temperature (25±2 °C) and light (12 h light/dark). Rats were acclimatized for 7 days before the start of the experimental work. They were fed with standard rat pellet diet and water ad libitum. Animal handling and experimental protocol were conducted according to the guidelines of the Institutional Ethical Committee (IEC).

Methods

***In vitro* cytotoxicity assay**

200µl cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. After 24 hrs, cell was treated with various concentrations 6.25µg/ml-100µg/ml of the RLX, CUM and RLX-CUM-NLC. Then, plates were incubated for 24 hrs at 37°C in a 5% CO₂ atmosphere [13]. After 24 h incubation, the cells were treated with 20 µl of MTT solution (0.5 mg ml⁻¹ in PBS) before being incubated for 3 h at 37 °C. Then, 100 µl of dimethyl sufoxide was added to completely dissolve the formazan crystals, absorbance was recorded at 570 nm and % cell viability was measured.

***In vivo* study**

Acute toxicity study

The acute toxicity study was conducted in accordance with Organisation for Economic Co-operation and Development (OECD) 423 Guideline for Testing of Chemicals.

Twelve animals (female rats) were randomly assigned into four groups (n=3 animals each), which were at four fixed doses (5, 50, 300 and 2000mg/kg of body weight) of CUM-RLX-NLC. One normal control with three animals was also taken in study. All treatments were given orally on the first day only. Rats were monitored for 14 days for any changes in general physical conditions such as appearance, body weight changes, behaviour and mortality. The body weight was measured thrice 0, 7 and 14 day using a table top electronic balance.

Haematological and biochemical analyses

Haematological parameters were analysed using an automated haematology analyser (Procan-P6800). The parameters measured were red blood cells, haemoglobin concentration, lymphocytes, white blood cells, and platelet count. Biochemical analysis was performed using a chemical analyser (Star 21 E114947). For hepatic function, the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin were evaluated. For renal function, the levels of blood urea nitrogen and serum creatinine were determined.

Histopathological Study

The organs, namely the liver, heart, spleen, lung, and kidney, were carefully excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study. The relative organ weight of each animal was calculated as follows: $\text{Relative organ weight} = (\text{organ weight (g)} / \text{body weight of the animal on sacrifice day (g)}) \times 100$. Tissue sections were cut and fixed in 10% formalin for at least 48 hrs. The fixed samples were placed in plastic cassettes and dehydrated using automated tissue processor. The processed tissues were embedded in paraffin and the blocks trimmed and sectioned were cut using a microtome (Spencer, US Pat 2-232-003). The tissue sections were mounted on glass slides using a hot plate (Remi) and subsequently treated in order with 100, 90, and 70% ethanol for two minutes each. Finally, the sections were rinsed with tap water and stained with haematoxylin and eosin for light microscopy.

Anticancer activity

Tumor Induction

These experimental rats were housed in standard polypropylene cages having 6 animals in each cage and they were randomly distributed into control and treated groups. A single dose of 75 mg/kg DMBA in 1 ml sesame oil was given to all animals p.o. Mammary carcinoma was confirmed by palpation. Rats were palpated weekly starting from 4th week after DMBA administration, to check for the tumor appearance. The first tumour appeared in the 4 months, after administration of DMBA while by 4.5 months tumour appeared in all the rats. The body weight of each animal in each group was also recorded weekly. Tumour size was measured by a vernier calliper (Aerospace) [14-17].

Experimental design

Animals (female wistar rats), aged 8-12 weeks, weighing (190 ± 20 g) were classified into 5 groups of 6 animals each.

Group I— Saline-treated rats (5 ml/kg) p.o. for 20 weeks.

Group II—DMBA induced rats only with single dose of 75 mg/kg DMBA in 1 ml sesame oil.

Group III— DMBA induced rats treated with Curcumin at the dose of 40 mg/kg by oral gavage daily after 14 weeks of tumor palpation until completing 20 weeks.

Group IV: DMBA induced rats treated with RLX at a dose of 15 mg/kg by oral gavage daily after 14 wk of tumor palpation until completing 20 weeks.

Group V: DMBA induced rats treated with RLX-CUM-NLC by oral gavages daily after 14 wk of tumor palpation until completing 20 weeks.

After the completion of dosing, female rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected through the orbital puncture of the experimental rats. Serum was separated for biochemical tests. The isolated tumor was subjected to endogenous antioxidant estimation. Tissues of breast were fixed in 10% formalin for the histopathological studies.

RESULTS & DISCUSSION

Preliminary *in vitro* cytotoxicity screening in human breast cancer cell lines

In vitro cytotoxic effect of RLX, CUM and RLX-CUM-NLC studied on MDA-MB-231 by MTT assay after 24 h of exposure showed that RLX, CUM and RLX-CUM-NLC produced dose-dependent decrease in the percentage viability of the cells (Figure 1-3) [18]. IC₅₀ values for RLX, CUM and RLX-CUM-NLC were found to be 45.37, 57.06 and 24.66 respectively on MDA-MB-231 cells. Among all three test materials, CUM-RLX-NLCs show effective cytotoxicity and may be considered potent anti-breast cancer agents due to their low IC₅₀ value on MCF-7 cells.

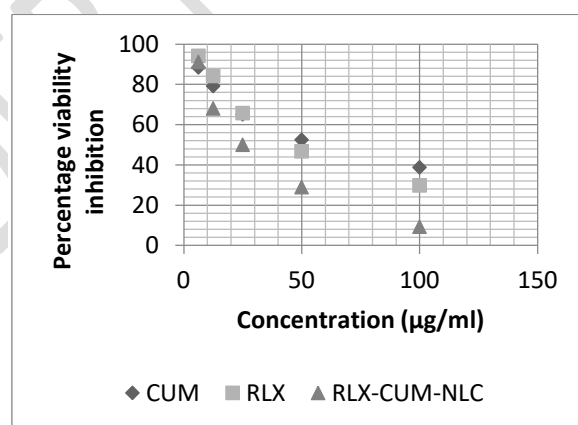


Fig.1: Cytotoxicity of RLX, CUM and its formulation RLX-CUM-NLCs at 24 h

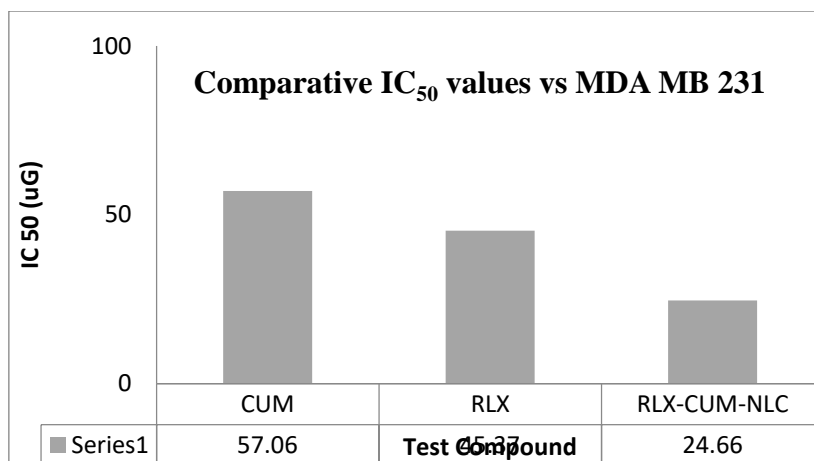


Fig. 2: Comparative IC₅₀ values vs MDA MB 231

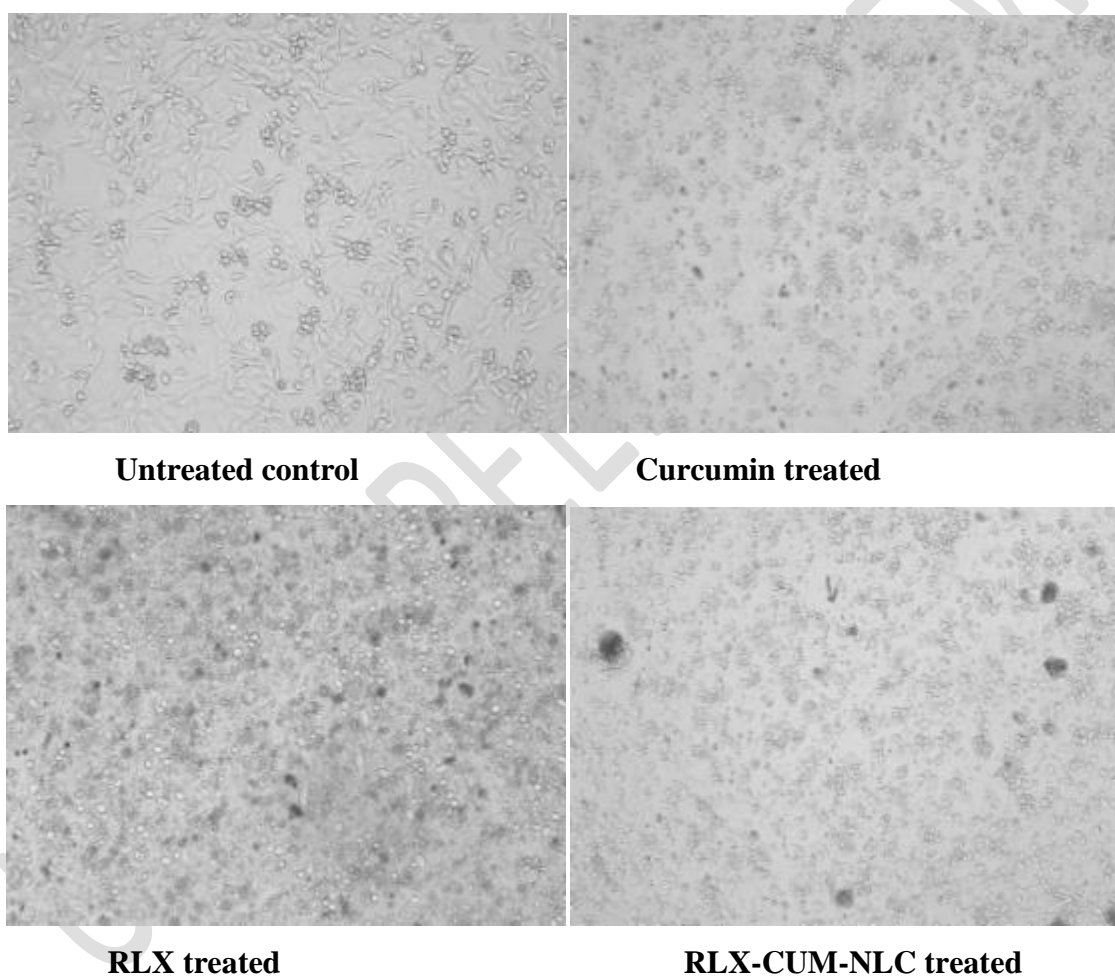


Fig. 3: Viable Cell of RLX, CUM and RLX-CUM-NLC treated MDA-MB-231 breast cancer cells

***In vivo* toxicity studies**

During the 14-day observation period, no significant weight loss was noted in all treatment groups till 300 mg/kg bw. Food and water consumption was found normal till 14 days as shown in table 1. There was no sign of behavioural abnormality in the surviving female rats.

So, depending on the current observed data of acute toxicity study, the final selected dose for further study were carried out using 1/20th of 300 mg/kg bw which was 15 mg/kg bw.

A single administration of RLX-CUM-NLCs did not cause any significant changes in the organ weight and body weight as shown in Table 2. Simultaneously the biochemical parameters were studied and summarized in Table 3.

Table 1: Food and water consumption

Groups	Food Consumption (gm/rat/day)			Water Consumption (ml/rat/day)		
	0	7	14	0	7	14
Normal control	11.45	12.85	13.15	8.5	9	8.8
5	12.55	15.25	17.85	8.3	8.5	9
50	12.85	13.91	14.85	8.5	8.3	9.2
300	11.67	13.35	14.25	8.3	8.8	8.9

Table 2: Body weight of rats treated with RLX-CUM-NLCs

Group	Dose (mg/kg)	Rat No.	Body Weight (gm)		
			0 Day	7 Day	14 Day
A	Normal Control	R1	201.22	203.78	206.24
	“	R2	198.99	200.33	202.19
	“	R3	200.00	202.54	204.18
B	5 mg/kg	R1	196.75	198.34	201.04
	“	R2	195.94	196.06	199.87
	“	R3	196.28	198.99	201.84
C	50 mg/kg	R1	198.79	200.50	202.20
	“	R2	195.65	196.48	199.06
	“	R3	197.63	199.46	202.24
D	300 mg/kg	R1	200.72	203.92	206.65
	“	R2	192.05	195.89	199.12
	“	R3	197.11	198.85	202.90

Table 3: Serum liver, renal and lipid profile of female rats treated with RLX-CUM-NLCs

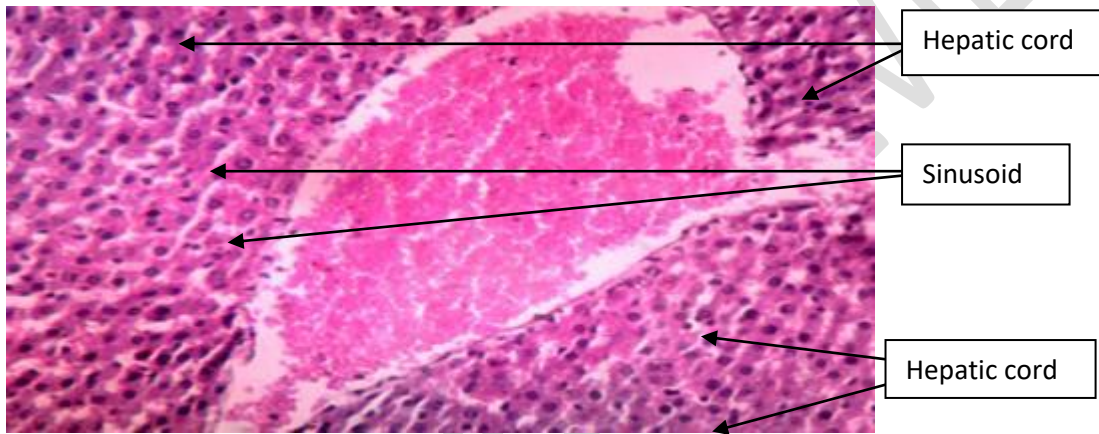
Groups	Test Parameter							
	ALT (IU/dL)	ALP(IU/dL)	AST (IU/dL)	Total Bilirubin (mg/dL)	BUN	Creatinine	TG	TC
Normal Control	45.93±1.82	101.4±5.18	95.02±4.13	0.4±0.05	15.82±1.93	0.92±0.03	93.47±6.49	109.3±13.51
5 mg/kg	47.29±1.82	108.2±8.5	99.16±2.8	0.49±0.0	17.4±1.52	0.91±0.0	93.98±9.1	104±9.78

	49		9	6		4	1	
50	51.01±3.	104.2±5.0	98.59±3.1	0.44±0.0	18.8±2.59	0.92±0.0	95.7±8.8	100.3±8.21
mg/kg	16	7	6	4		2		
300	53.74±2.	110.6±7.6	97.42±3.9	0.45±0.0	19.4±1.14	0.9±0.03	98.92±9.1	96±8.94
mg/kg	02	7	7	4			2	

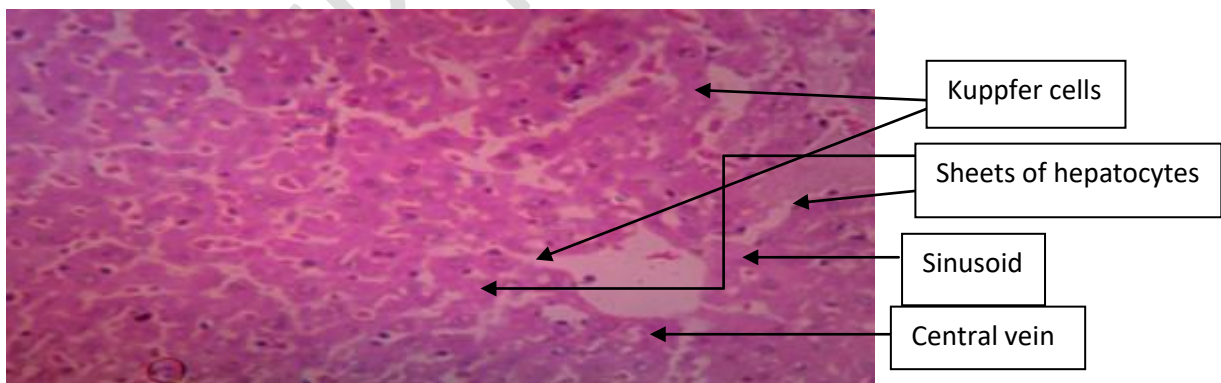
Histological changes

Liver

Control rats show normal architecture of the liver with hepatocytes radiating from the central vein, sinusoidal space; RLX-CUM-NLCs at 5,50,300 mg/kg bw treated rats showed normal architecture but 2000mg/kg bw treated rats show moderate parenchymal cells, dilatation of sinusoidal space with severe inflammatory cell infiltration; H&E (10X) shown in figure 4.



Normal Control



5 mg/kg bw

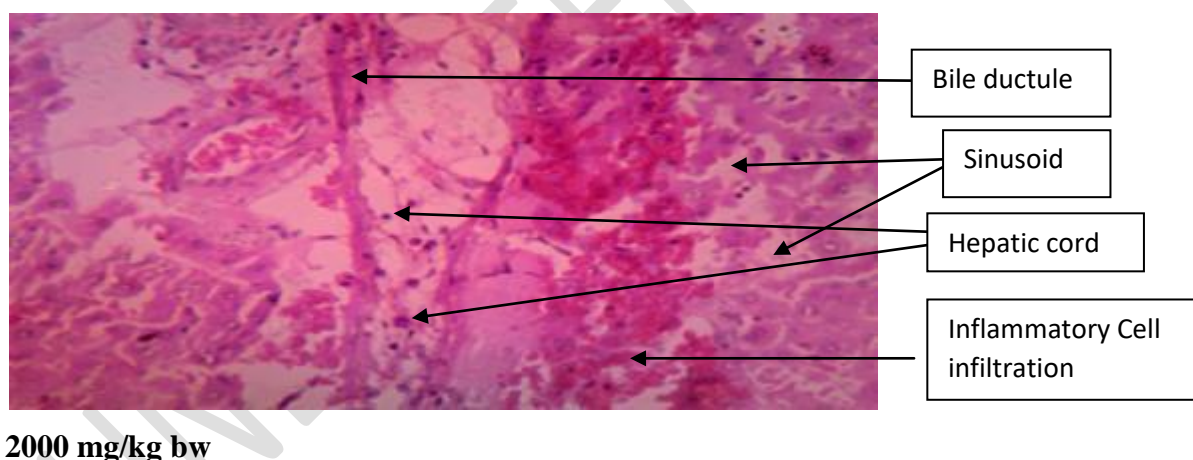
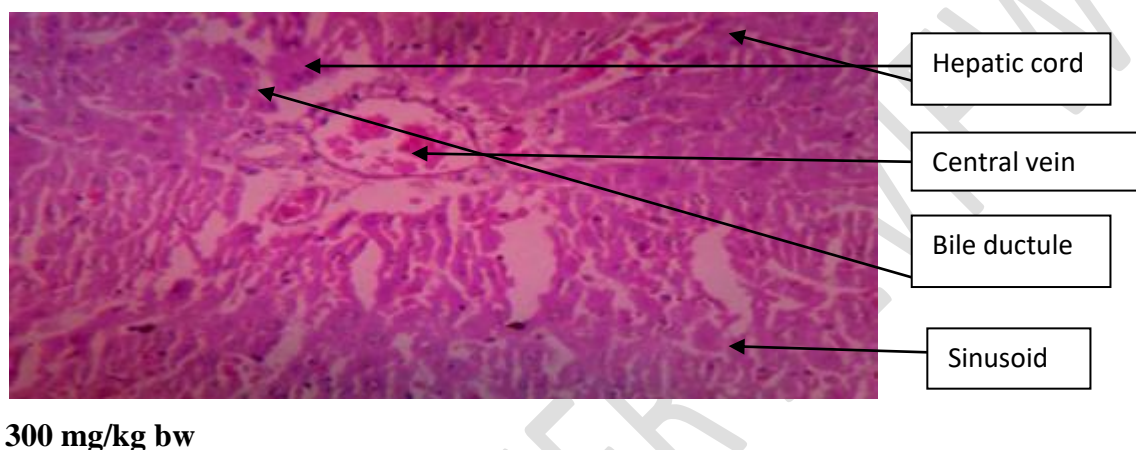
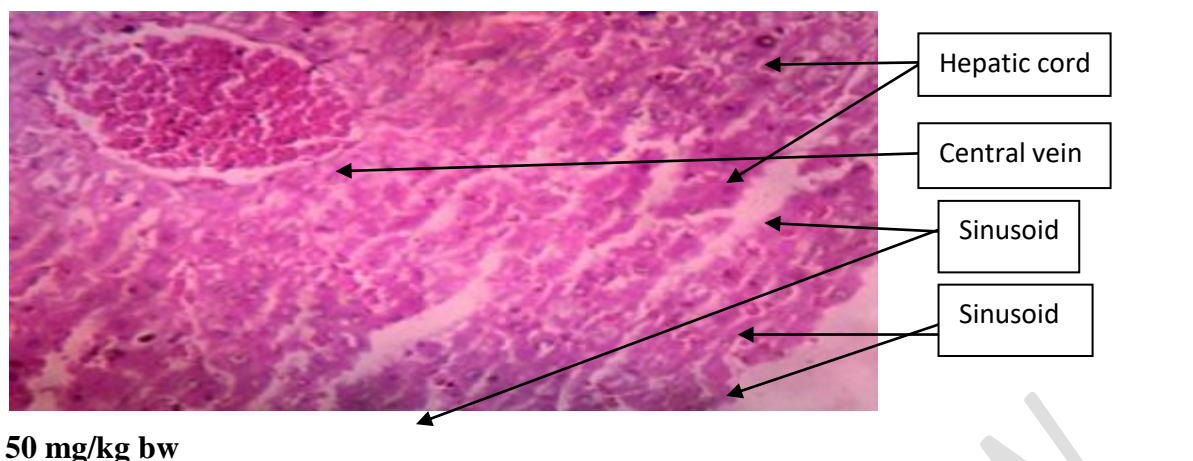
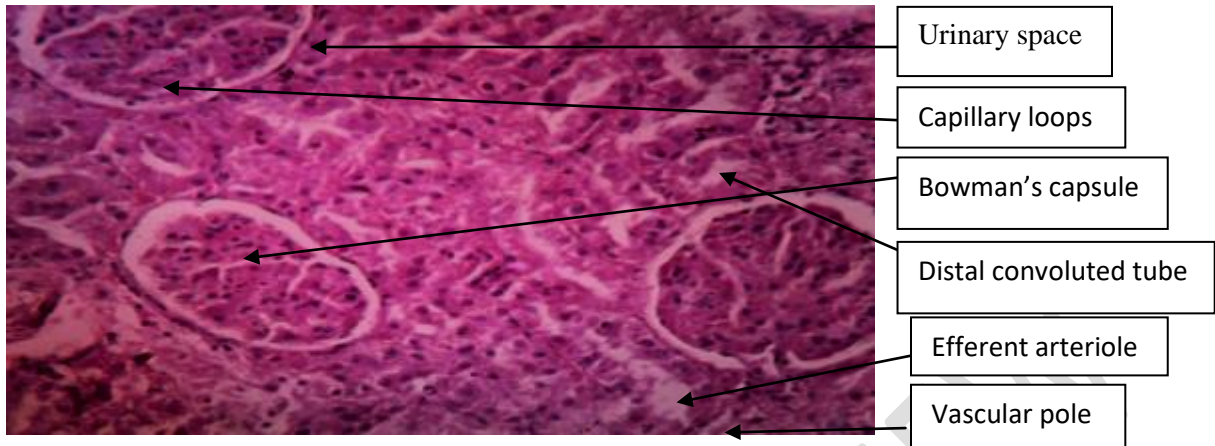


Fig. 4: Histology of Liver in actue toxicity studies

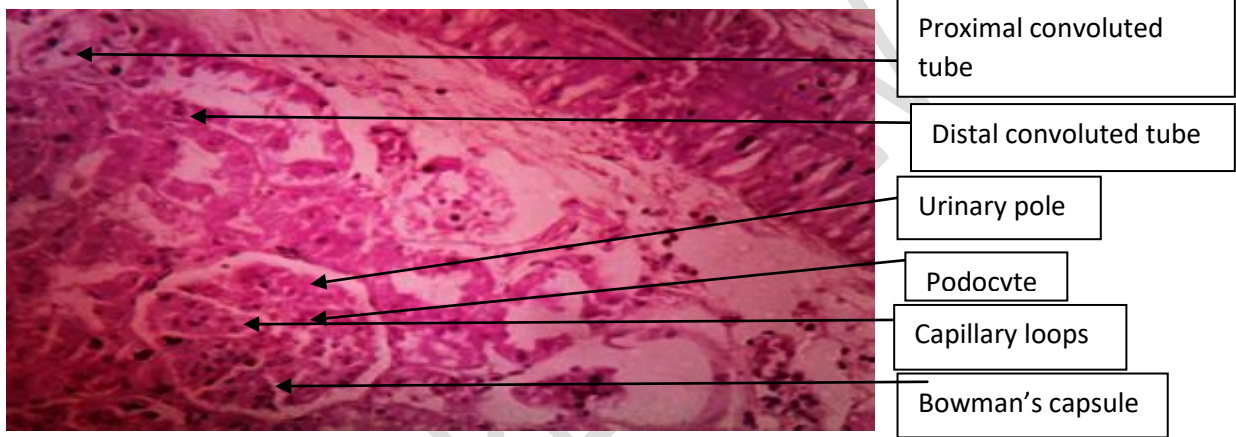
Kidney

H&E-stained kidney sections of normal control group looked normal with clearly visible glomeruli, basement membrane of the capsular wall. The interstitial appeared normal and with no infiltrations. In 5, 50 and 300 mg/kg bw RLX-CUM-NLC treated rats, some of the proximal and distal convoluted tubules showed hypertrophic cells, glomeruli with Bowman's space and capillary tufts, which appeared normal and vacuolization and in 2000 mg/kg bw treated rats showed a severe distortion of the glomerular architecture, with atrophic and

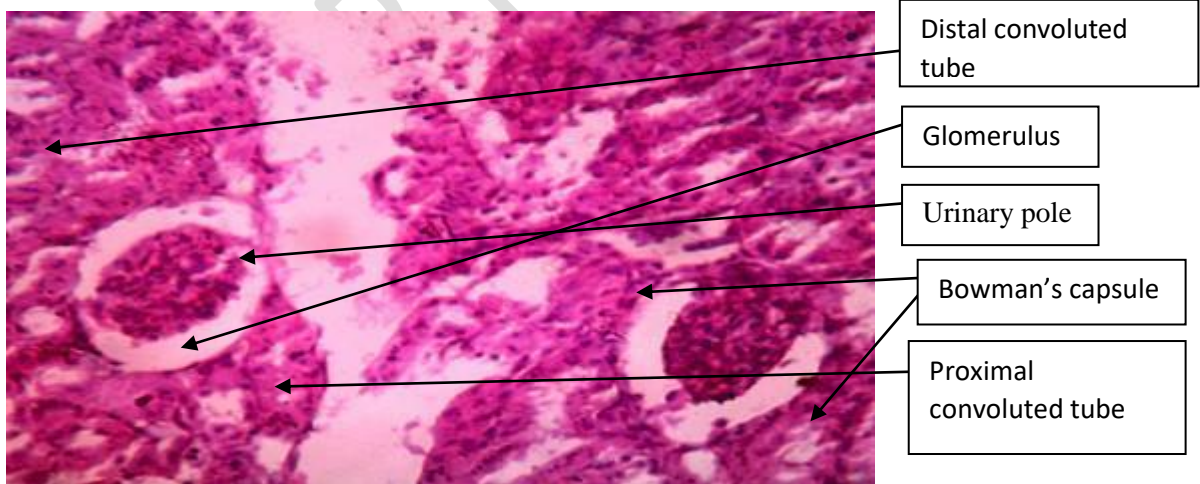
necrotic changes, pyknotic nuclei as well as infiltrations of mesangial spaces and interstitial shown in figure 5.



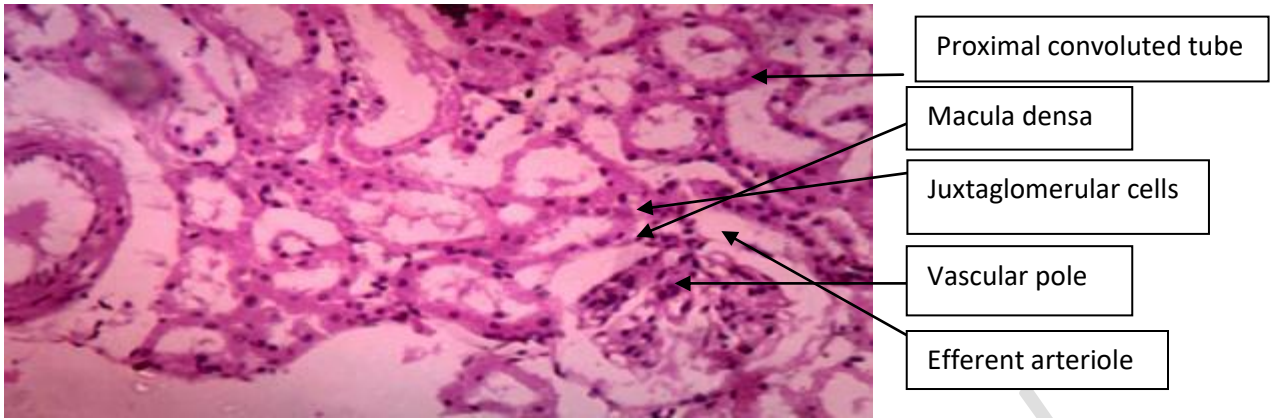
Normal control



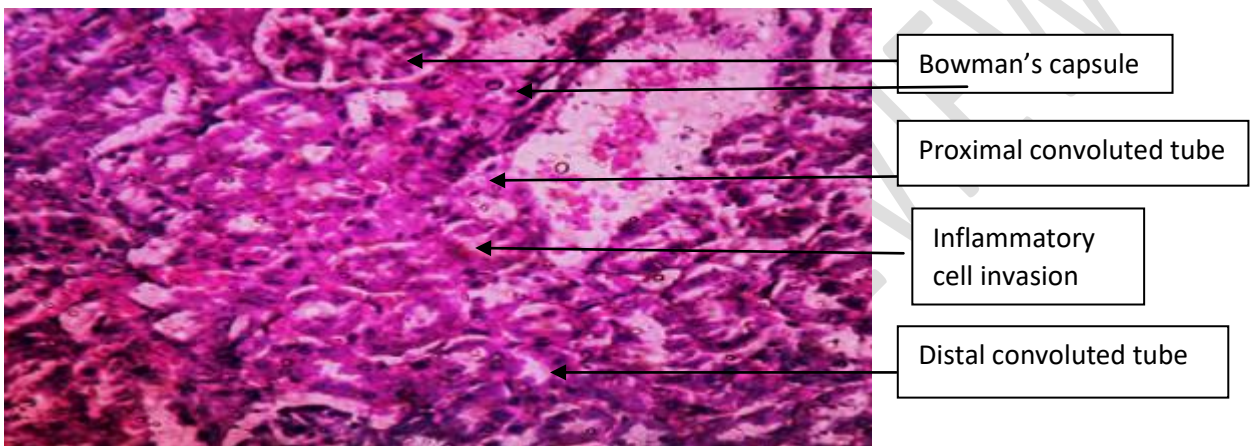
5mg/kg bw



50 mg/kg bw



300 mg/kg bw

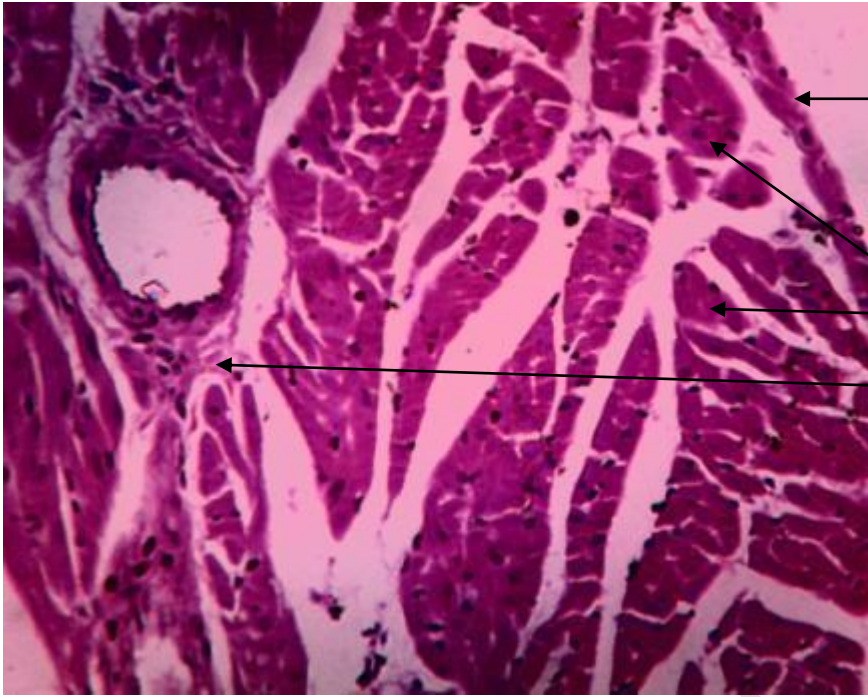


2000 mg/kg bw

Fig.5: Histology of Kidney in acute toxicity studies

Heart

Histological analysis of rat heart with H and E staining Normal control; most cells are elongated and rod shaped with striated muscles. The 300 mg/kg bw treated rats showed rod shaped cells which are intact and except few cells all the cells are normal but in 2000 mg/kg bw treated rats cell swelling, striated muscles and blood vessels seen ((Figure 6). 30 min ischemia followed by 45 min reperfusion; cell swelling and increased congested blood vessels were observed.

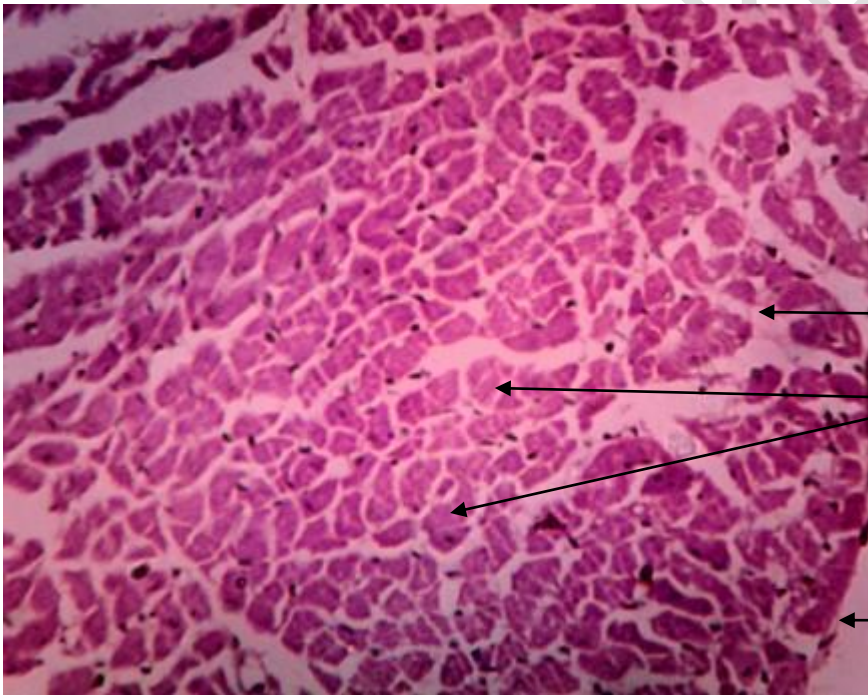


Endothelium

Striated muscle cells

Intercellular spaces

Normal control

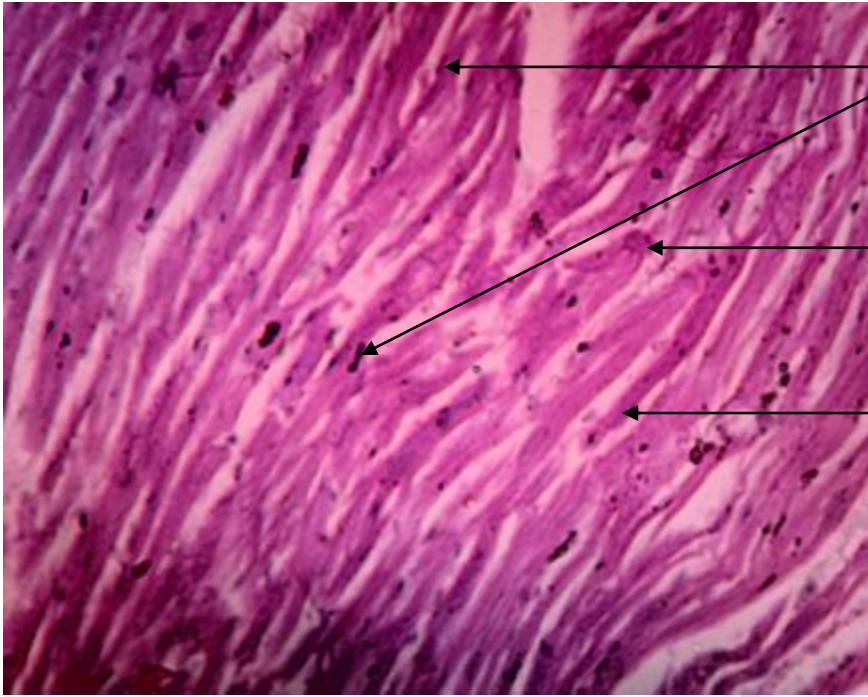


Endomysium

Striated muscle cells

Endothelium

5 mg/kg bw

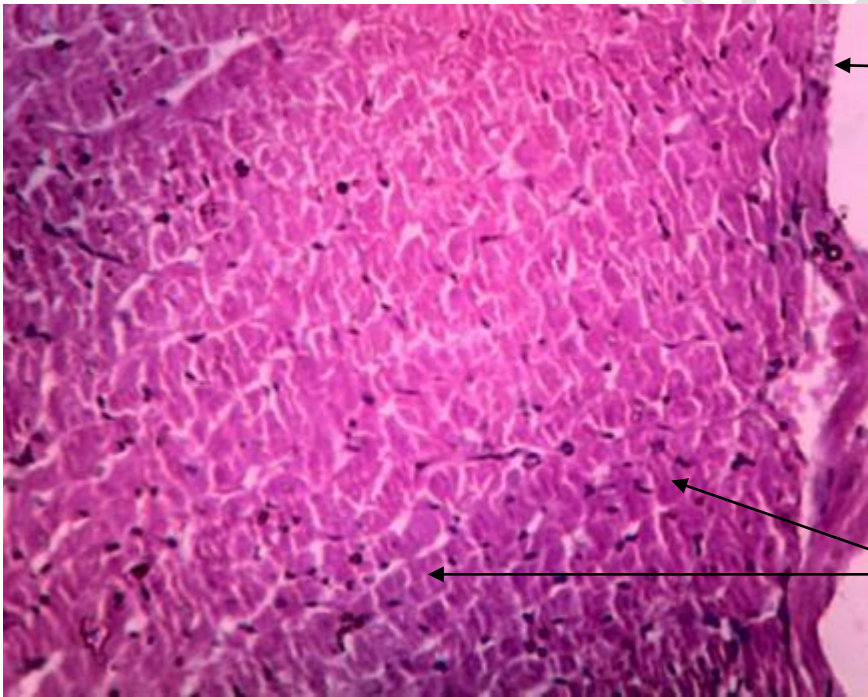


Heart muscle fibres

Nuclei

Longitudinal fibers

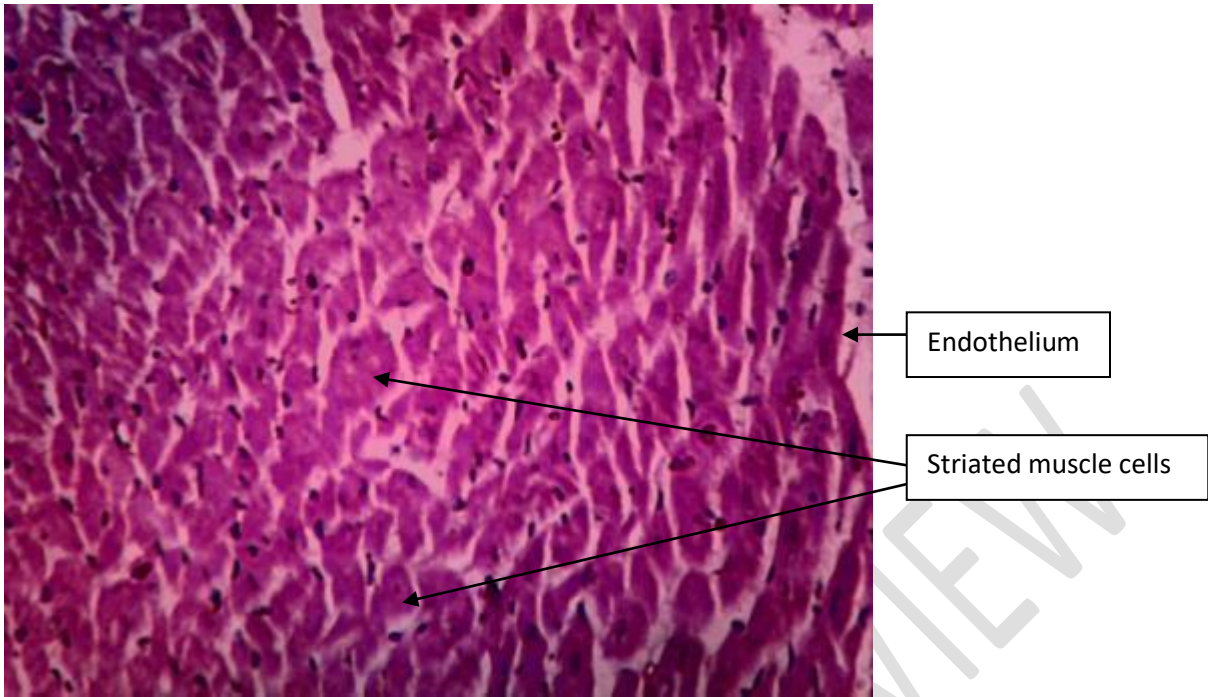
50 mg/kg bw



Endothelium

Striated muscle cells

300 mg/kg bw

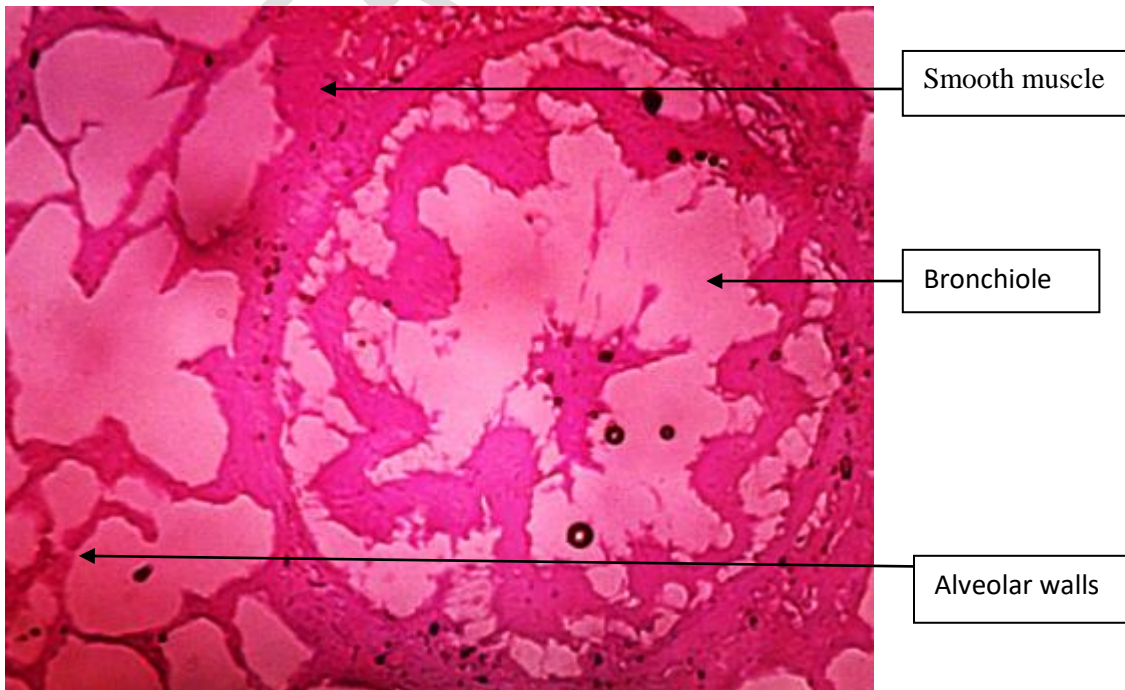


2000 mg/kg bw

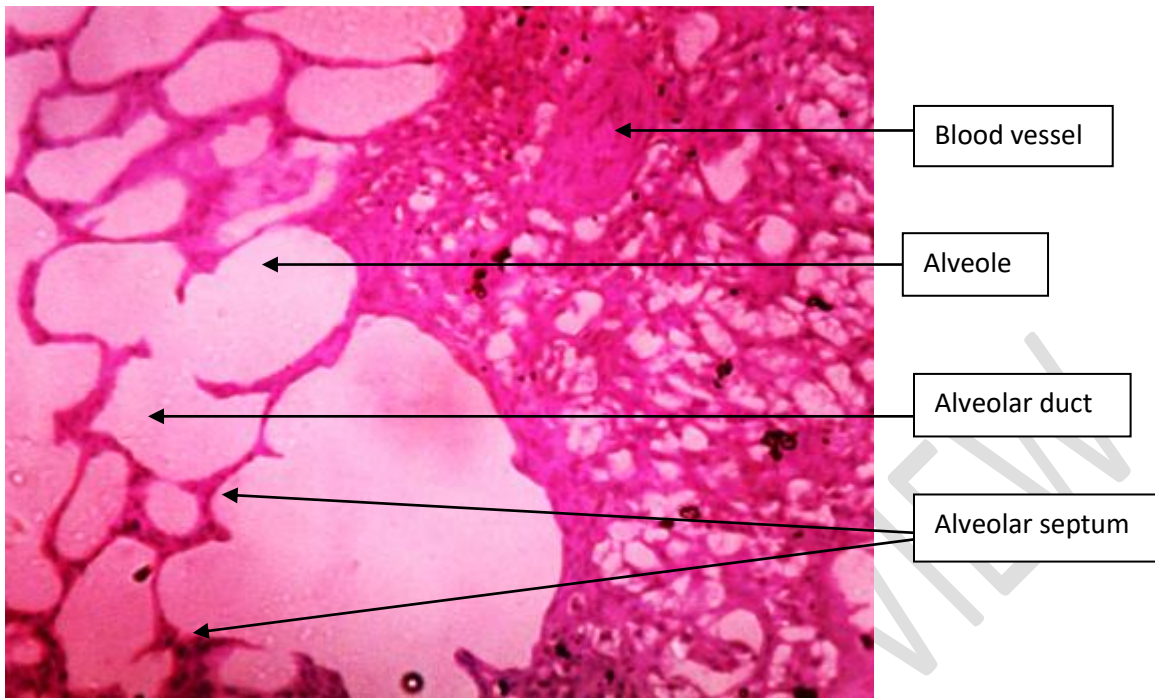
Fig. 6: Histology of Heart in acute toxicity studies

Lungs

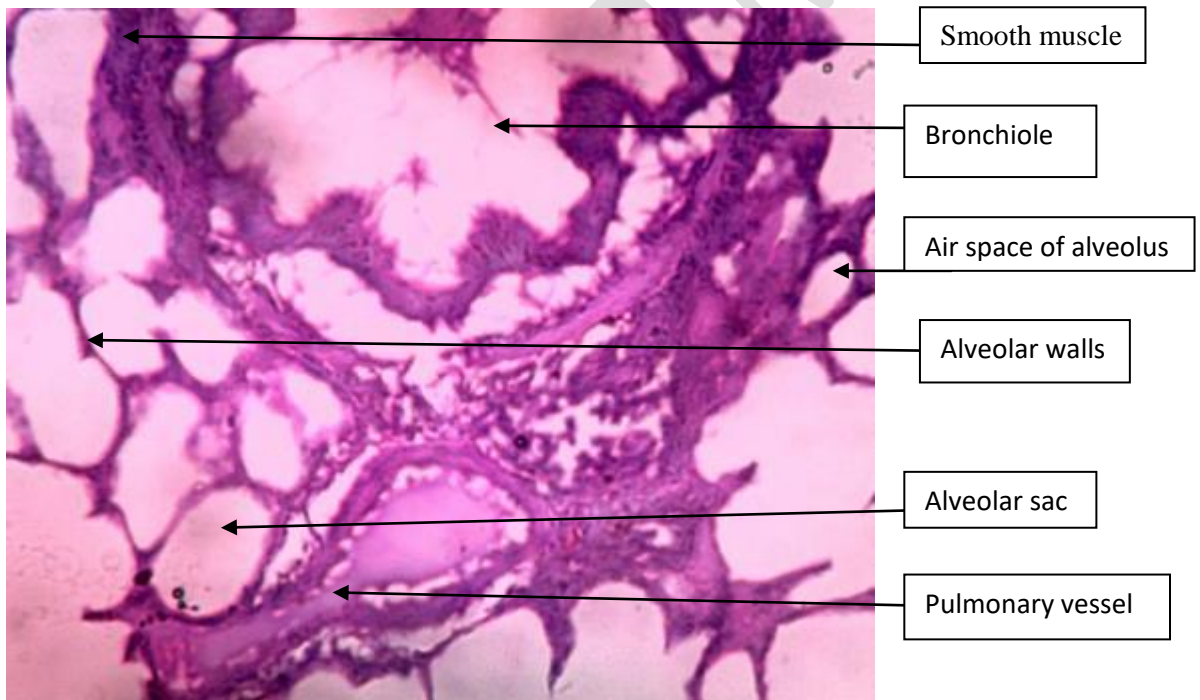
Photomicrograph showed the normal histological structure of the epithelial cells in normal control rats (Figure 7). The 5, 50, 300 mg/kgbw treated bw group showed bronchiole, alveolar sac, pulmonary vessels, septum and 2000 mg/kgbw rats showed intensive contraction of alveoli, diffuse accumulation of inflammatory cells in the pulmonary parenchyma compressed filled with necrotic debris



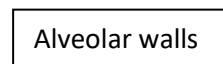
Normal control

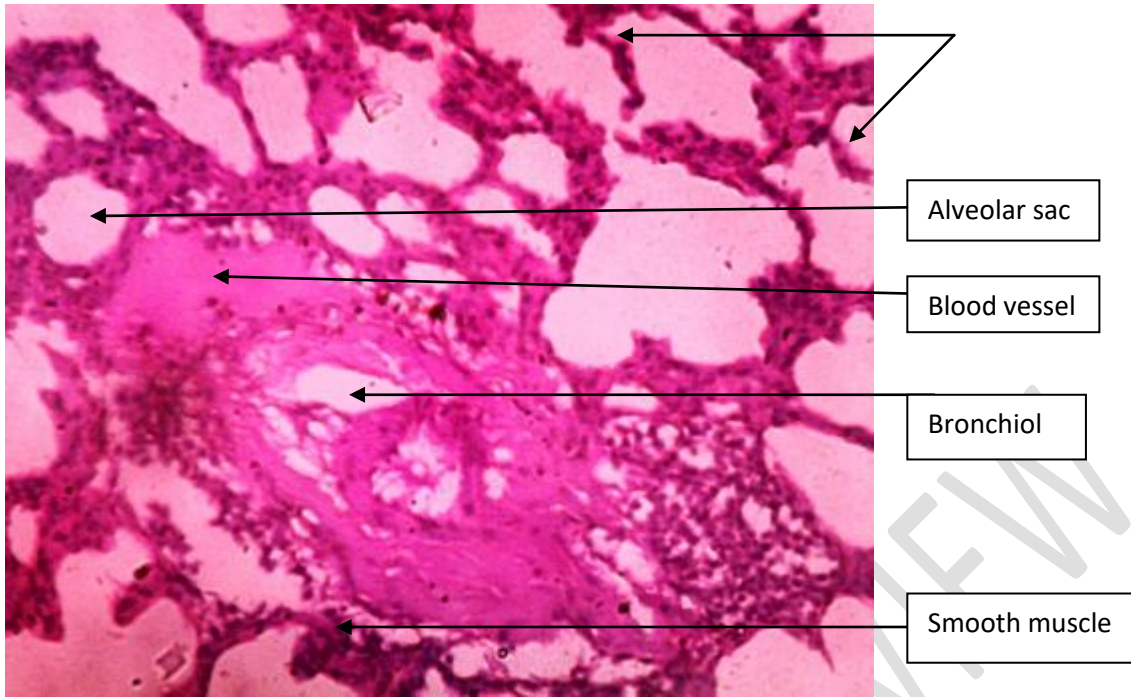


5 mg/kg bw

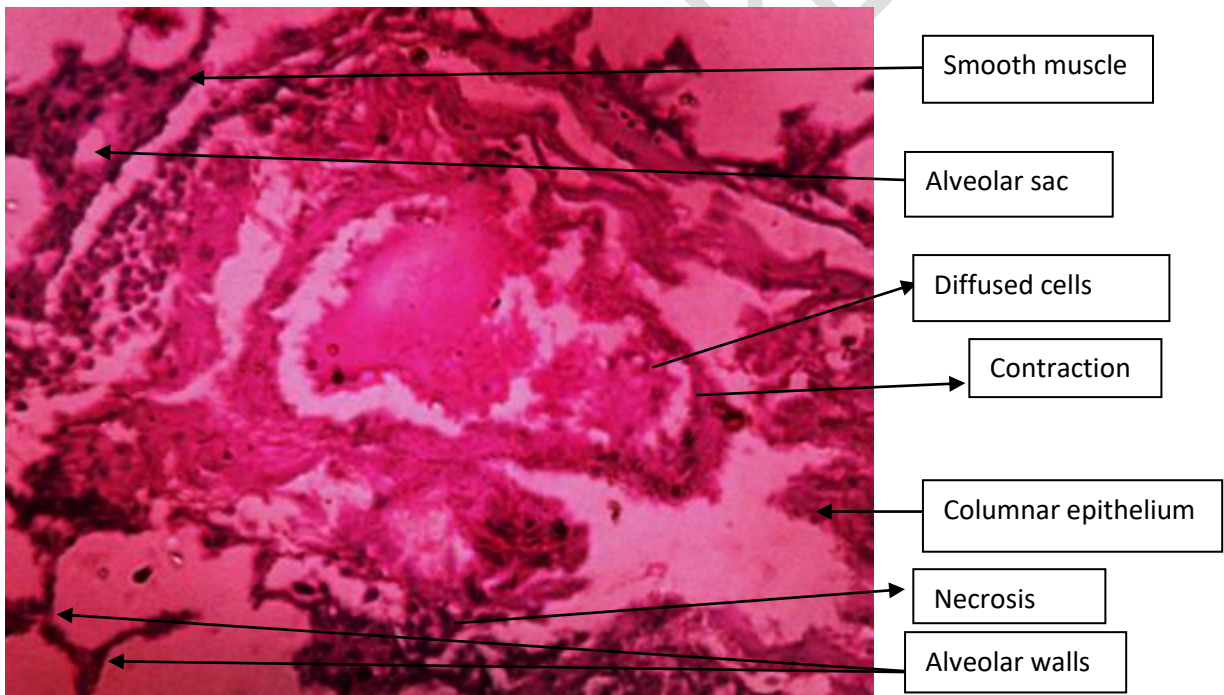


50 mg/kg bw





300 mg/kg bw

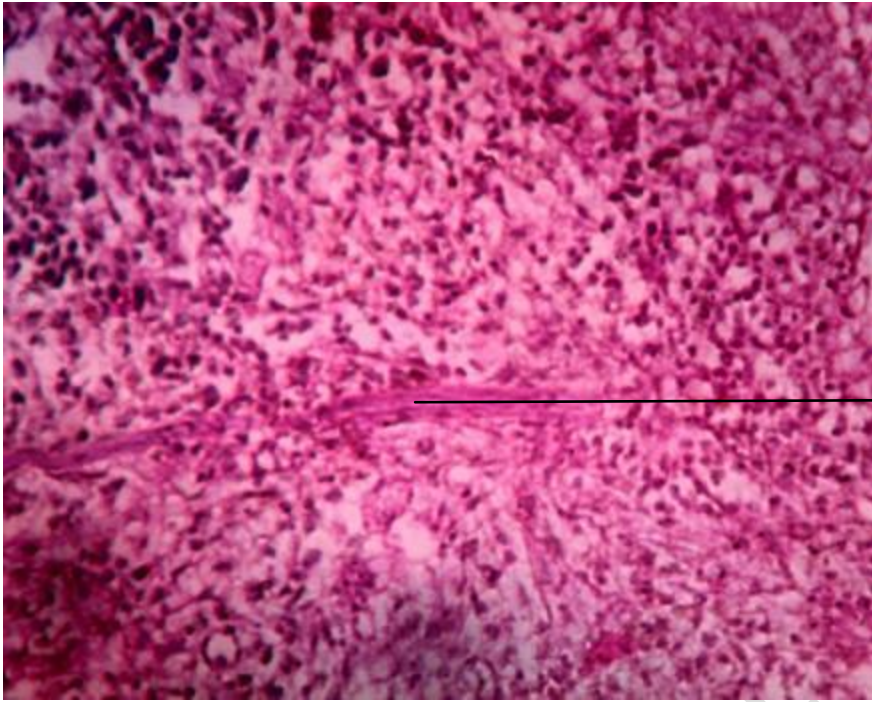


2000 mg/kg bw

Fig.7: Histology of Lungs in actue toxicity studies

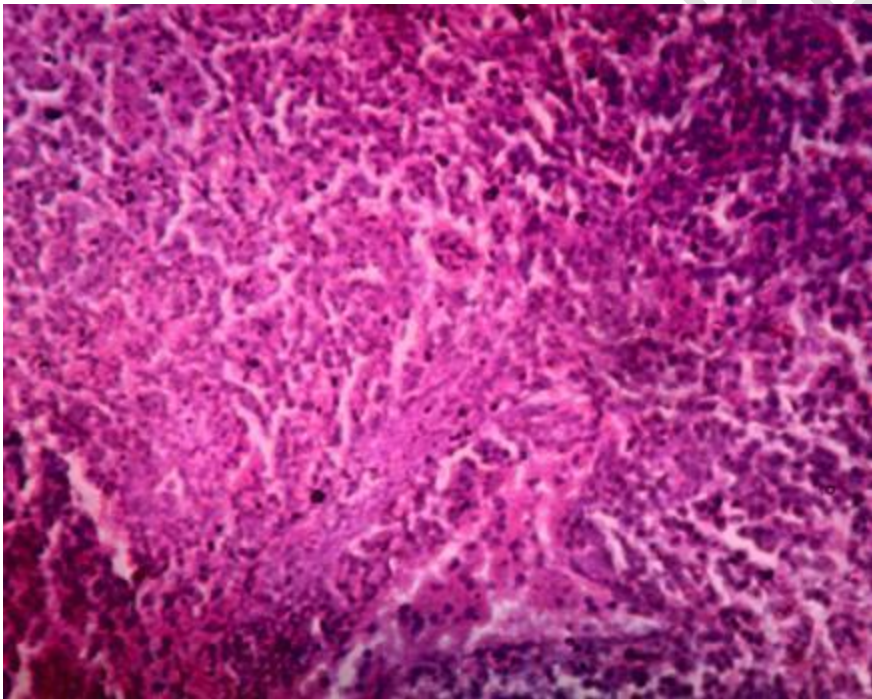
Spleen

Photomicrographs of spleen tissues of a rat showed in Normal, 5, 50, 300 mg/kg bw showed periarterial lymphoid sheath, trabecular artery and 2000 mg/kgbw treated rats showed lymphoid cell atrophy, reticular cells hyperplasia in H&E stain at 10X (Figure 8).

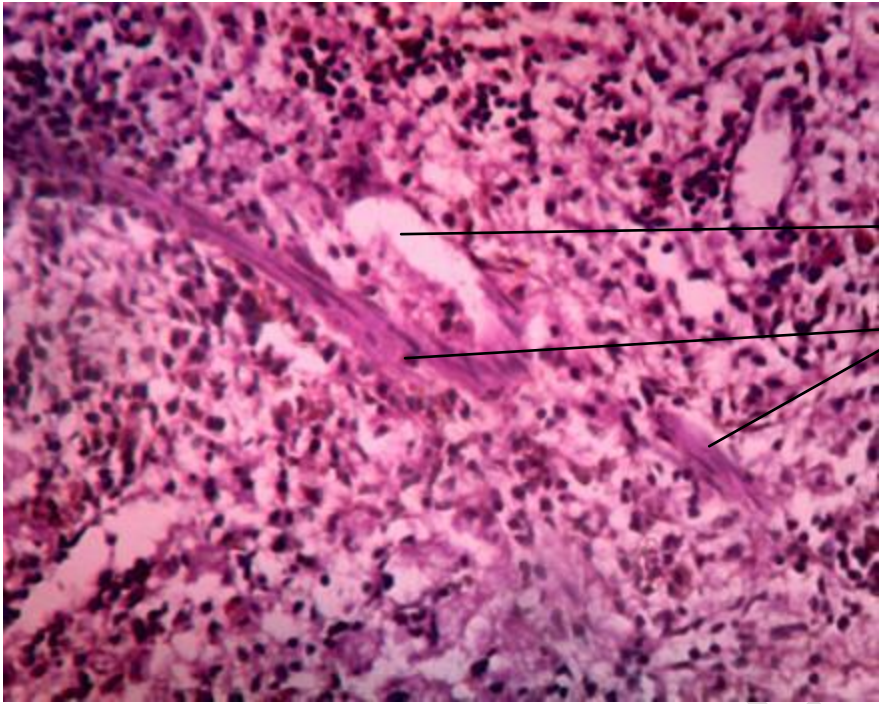


Periaarterial
lymphoid sheath

Normal control



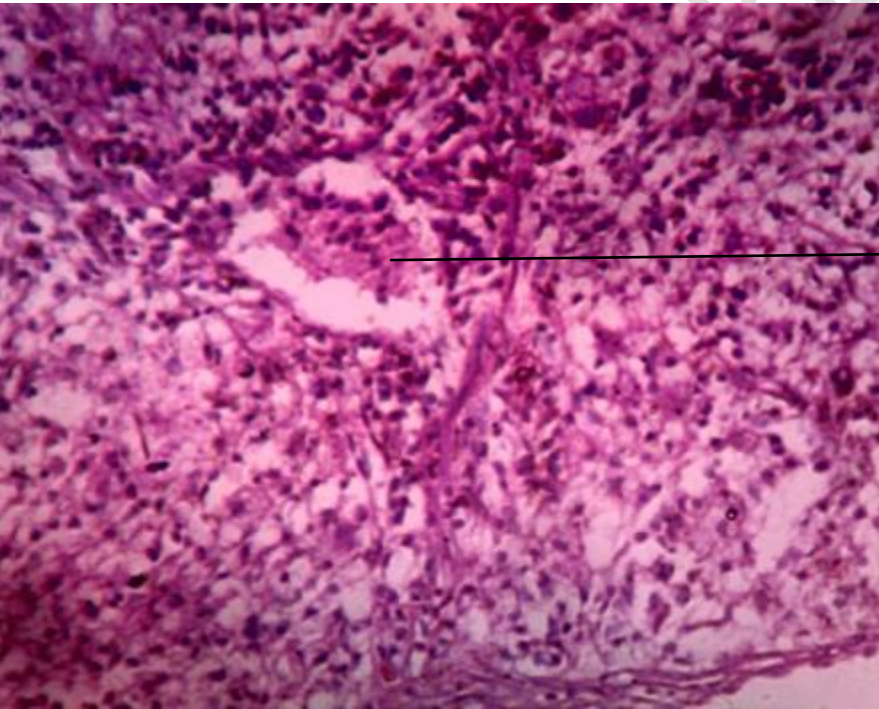
5 mg/kgbw



Vacuole

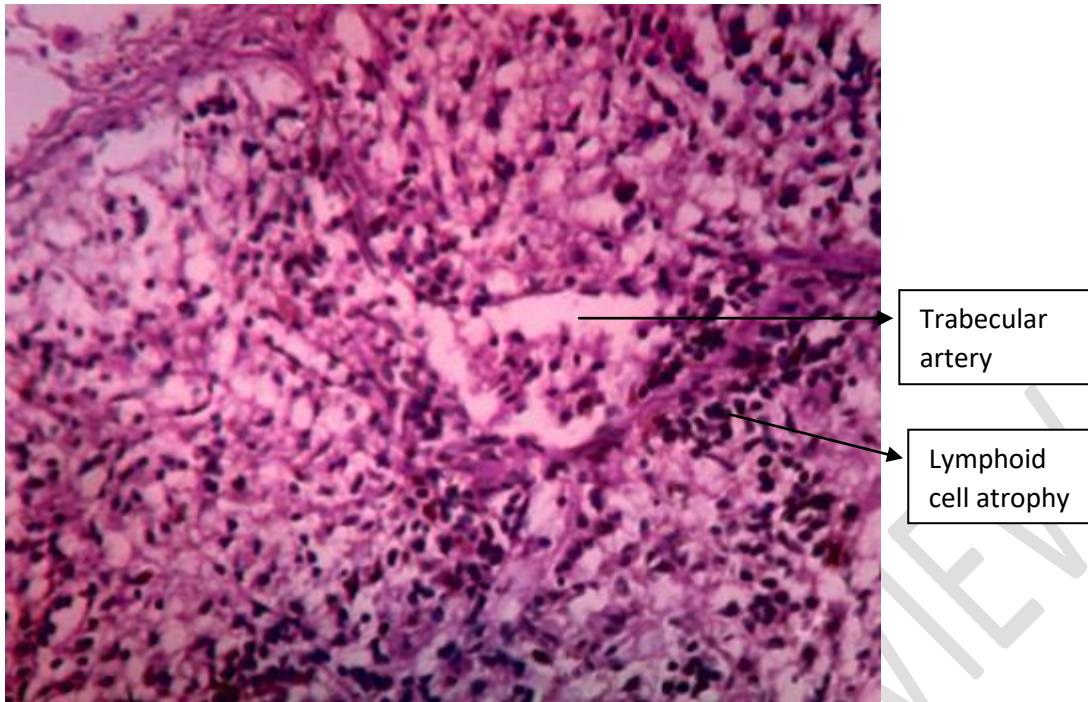
Periarterial
lymphoid sheath

50 mg/kg bw



Trabecular
artery

300 mg/kg bw



2000 mg/kg bw

Fig.8: Histology of Spleen in acute toxicity studies

***In vivo* study**

Body weight changes

The body weight changes of animals in DMBA induced cancers and treated with RLX, CUM and RLX-CUM-NLC was presented in graph.

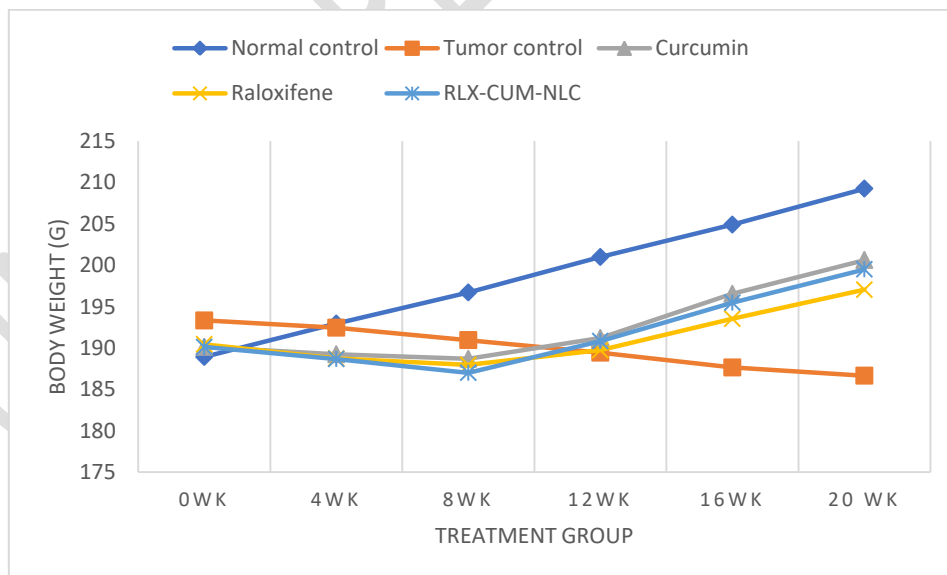


Fig.9: Body weight changes in RLX, CUM and RLX-CUM-NLC

Tumor volume is a potential biomarker for breast cancer development. All the treatments group showed significant ($p < 0.05$) reduction in tumor volume as compared to Tumor control.

The RLX-CUM-NLC-treated group showed significant ($p < 0.05$) inhibition in tumour volume as compared to other treatment groups (Figure 9 & 10).

Tumor volume was $82.06 \pm 7.311 \text{ mm}^3$ for DMBA induced group, $31.72 \pm 4.748 \text{ mm}^3$ for CUM, $20.22 \pm 10.583 \text{ mm}^3$ for RLX and 6.748 ± 1.567 for RLX-CUM-NLC-treated which was significantly ($p < 0.05$) low as compared to tumor control on 20th week. As expected, animals treated with RLX-CUM-NLC-treated showed a significant ($p < 0.05$) reduction in tumor weight of ($0.12 \pm 0.10 \text{ g}$) as compared to DMBA control animals (1.81 ± 0.31).

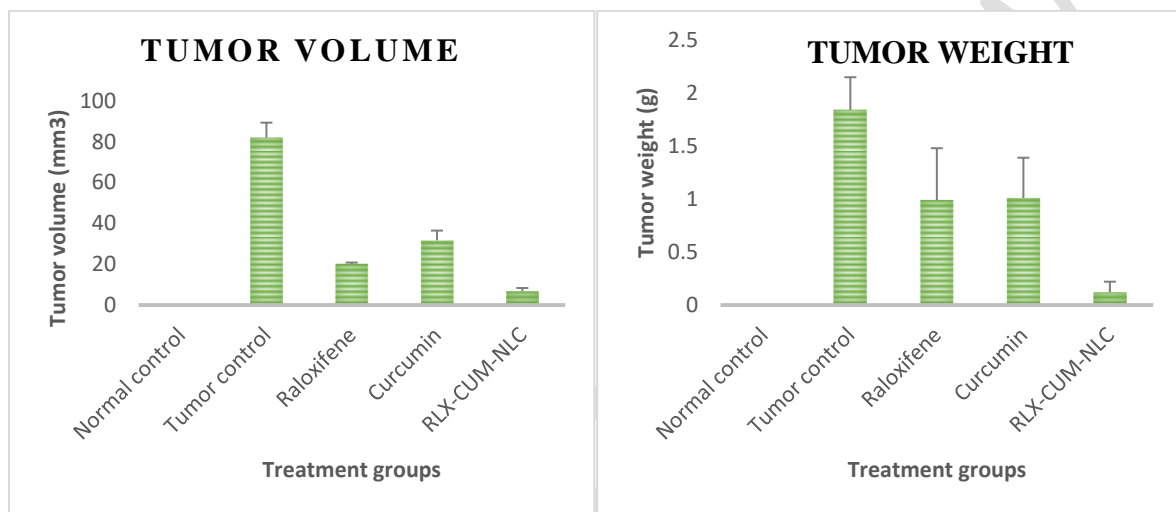


Fig.10: Tumour volume & weight RLX, CUM and RLX-CUM-NLC treated groups

On the basis of assessment parameters and the results obtained from the tumors excised from all the treated rats, it was found that the tumor volume, tumor weight, tumor burden, tumor yield and tumor incidence was found to be increased in the DMBA-treated groups (Tumor control) when compared with the treatment groups. There was a significant decrease in the below mentioned tumor parameters in animals administered with RLX, CUM and RLX-CUM-NLCs. RLX-CUM-NLCs demonstrated a rapid and significant decrease in tumor volume, tumor weight, and tumor burden, and tumor incidence when compared with other treatment groups. In the tumor control group CUM treated group, the tumor incidence rate was 100%; however, in the group treated with RLX, the tumor incidence rate was markedly reduced to 83.33% and in RLX-CUM-NLCs treated group its was reduced to 50%. These data suggest that RLX-CUM-NLCs treated could prevent the chemically carcinogenesis induced by DMBA-treatment (Table: 4). Oxidative stress also plays an important role in progression of cancer breast. This study was conducted to compare the levels of superoxide dismutase (SOD), GSH and malondialdehyde (MDA) in breast cancer rats (Table: 5). In contrast, haematological study was done to study the complete blood profile in rats (Table 6).

Table 4: Effect of RLX, CUM and RLX-CUM-NLCs on tumor parameters

Group No.	Treatment	Tumor yield	Tumor burden	Tumor incidence
I	Normal control	-	-	-
II	Tumor control	4.167	4.167	100
III	Curcumin	2.830	2.83	100
IV	Raloxifene	2.167	2.60	83.33
V	RLX-CUM-NLC	1.167	2.33	50.00

Table 5: Effect of RLX, CUM and RLX-CUM-NLCs on oxidative stress

Group No.	Treatment	SOD (Unit/mg tissue)	LPO (nmol MDA/mg tissue)	GSH (nmol/mg tissue)
I	Normal control	45.94±10.46	23.27±1.389	1.980±0.034
II	Tumor control	7.93±4.891	68.61±2.495	0.481±0.034
III	Raloxifene	30.48±7.409	40.91±1.448	1.301±0.033
IV	Curcumin	27.28±5.481	49.02±2.186	1.219±0.018
V	RLX-CUM-NLC	38.28±10.970	29.63± 1.889	1.548±0.045

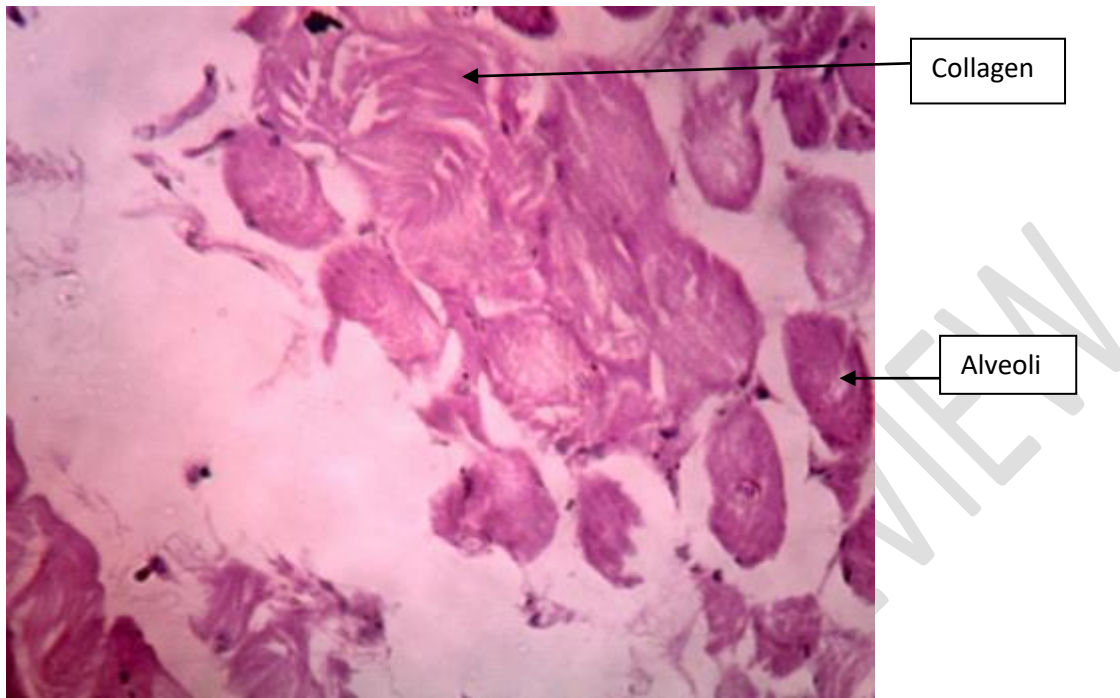
Table 6: Effect of RLX, CUM and RLX-CUM-NLCs on haematological parameters

Group No.	Treatment	Hb (gm/dL)	RBC (106/ μ L)	WBC (103/ μ L)	DLC (%)	TLC (%)	PLATELET S (103/ μ L)
I	Normal control	14.62±1.775	8.12±0.186	9.02±0.174	44.02±5.373	67.47±4.068	469.33±10.671
II	Tumor control	5.92±0.663	4.12±0.518	4.29±0.644	14.56±1.847	47.49±4.445	414.83±7.653
III	Raloxifene	12.37±0.692	7.86±0.172	7.95±0.235	38.67±2.333	59.25±2.136	455.00±17.274
IV	Curcumin	10.75±1.169	6.58±0.913	7.44±0.725	34.65±2.489	59.37±5.403	442.67±9.606
V	RLX-CUM-NLC	11.68±0.278	7.31±0.652	7.61±0.351	38.99±3.396	69.43±8.375	463.17±463.17

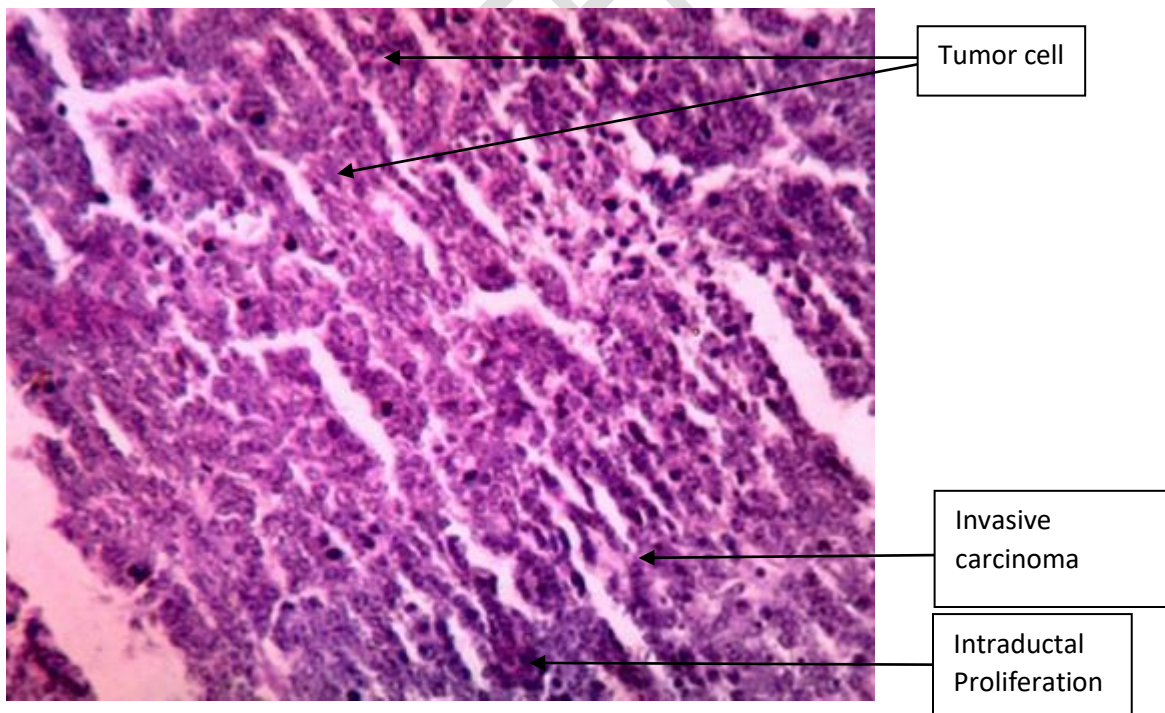
Histology

Histopathology of tumor control group exhibited a distortion in the normal architecture of rat mammary gland with tumor stroma, invasive carcinoma, loss of tubuloalveolar pattern was observed along with the presence of large epithelial cells, Intraductal necrosis, Malignant ductile, Basement membrane and myoepithelial cells. Treatment with CUM was not significant in comparison with the normal control as observed by the distorted architecture of

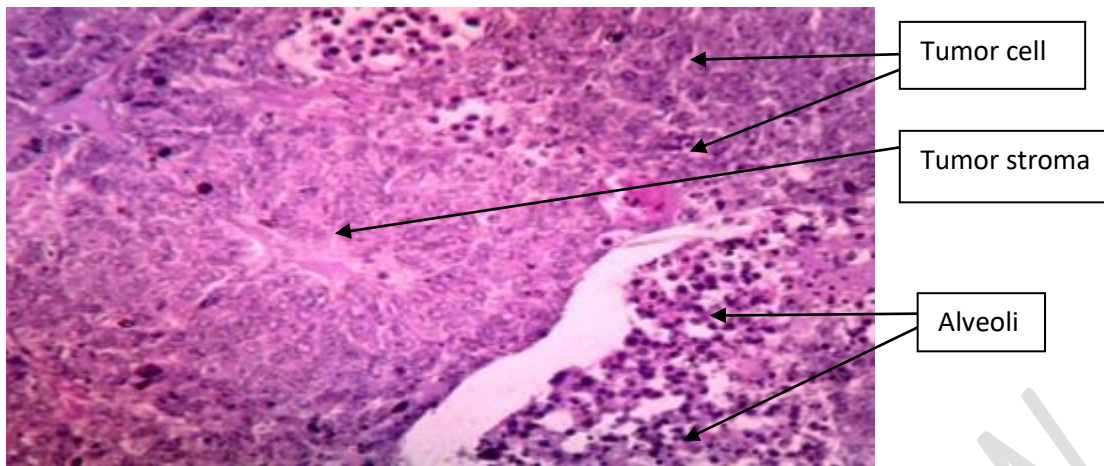
the epithelial cells. In contrast, histopathology of the RLX treated group showed a restoration in the tubuloalveolar pattern (Figure 11). Moreover, RLX-CUM-NLC treatment restored the altered shape of the alveolar cells.



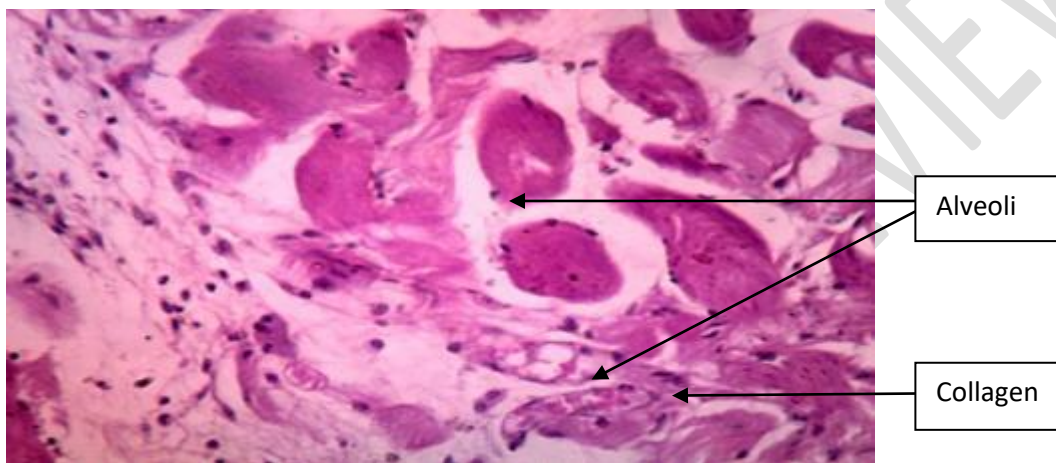
Normal control



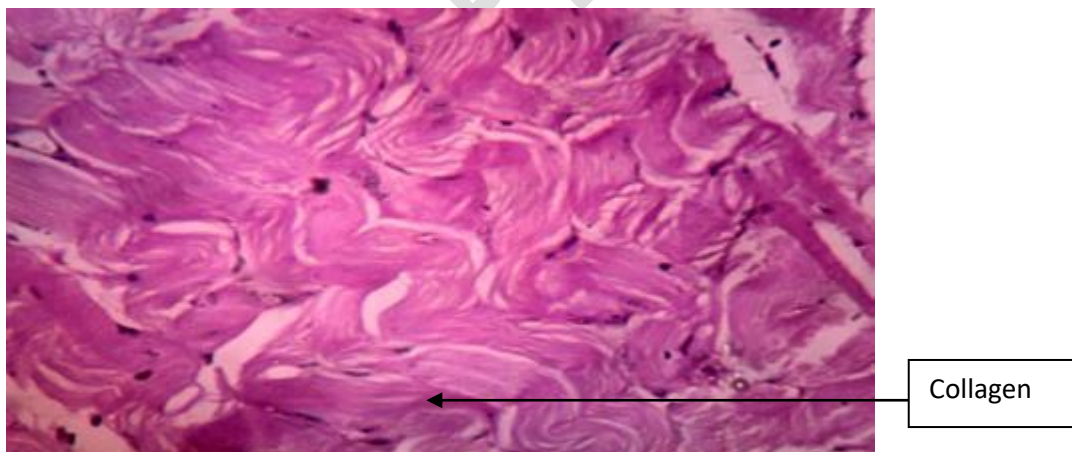
Tumor control



CUM treated



RLX treated



RLX-CUM-NLC Treated

Fig.11: Histopathology of breast cancer tissue

In vitro studies have shown that NLCs encapsulated RLX-CUM possess cytotoxic potential against breast cancer cells and when given in Acute toxicity study, it is found suitable for oral administration without causing acute toxicity in rats. However, the *in vivo* oral efficacy of RLX-CUM -NLCs was compared with RLX and CUM in the breast cancer animal model.

Also, both RLX and CUM have shown similar *in vitro* cytotoxicities on triple negative MCF-7 cell lines, it is valuable to compare the advantages of slow *in vivo* release of NLC encapsulated and free RLX and CUM against triple negative breast cancer cells. Further, in *in-vivo* study, RLX and CUM treatment decreased the number of tumor. We worked on DMBA-induced rat mammary carcinoma model. The tumor incidence of our study were similar to other studies with the DMBA, we found tumor after 14 weeks of induction and incidences of 100%. In RLX treated group, RLX inhibits tumorigenesis effect of the chemical carcinogen DMBA and the incidence of breast experimental cancer reduced to 83.33 % but RLX-CUM-NLCs showed potent anticancer effect with 50% incidence. RLX-CUM-NLCs treatment did not show any signs of toxicity on the animals according to their food consumption, body weight and activity levels compared with controls till 300 mg/kg bw.

CONCLUSION

In the present study, no toxicity was observed in histopathology of lung, heart, spleen liver and kidney in the groups that received RLX-CUM-NLCs treatment till 300 mg/kg bw. Slides of 2000 mg/kg treated rats were also presented showing toxicity in various organs. Oxidative stress is a key mechanism inducing cytotoxicity through enhancing the production of MDA accompanied by depletion of GSH in tissues; these are considered the most important indexes of antioxidant activities. Significant reduction of oxidative stress and haematological parameters were observed in treatment groups. The levels of oxidative stress markers such as level of LPO were substantially increased by DMBA treatment in control group. However, in treatment group RLX-CUM-NLCs significantly restored the required LPO level. Oxidative stress as indicated by decrease in (GSH) and (SOD) activity compared with the tumour control group values. The present study reveals that the RLX-CUM-NLCs exert chemo preventive effect by suppressing the tumour burden and restoring the activities of marker enzymes on DMBA induced breast cancer in rats.

Ethical Statement: All animal experiments were performed with prior permission of Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. 1824/PO/Rc/S/15/CPCSEA). Protocol approval number: PBRI/IAEC/08-22/031.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement

of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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