Chapter 8

Toxicity studies

8.1. Introduction:

8.1.1. Acyclovir:

Acyclovir is an antiviral drugs which is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV (O'Brien JJ et al 1998). The most frequent adverse effects reported during the second year by 390 patients who elected to continue daily administration of 400 mg (two 200 mg capsules) 2 times daily for 2 years were headache (1.5%), rash (1.3%), and paresthesia (0.8%). Adverse events reported by 329 patients during the third year include asthenia (1.2%), paresthesia (1.2%), and headache (0.9%). It was also reported as abnormal urogenital syndromes which include nephrotoxicity and elevated creatinin. Beside the drug, the formulation contains higher percentage of surfactant. To evaluate the safety of various formulation as compared to the commercial available dosage form, toxicity studies was carried out

8.12. Efavirenz:

Efavirenz is a non-nucleoside reverse transcriptase (RT) inhibitor of human immunodeficiency virus type 1 (HIV-1).As the metabolism of the efavirenz was taken place in liver; hepatoxicity is a serious concern on its uses. To evaluate the safety of the formulation histopathology study of liver was carried out. Here also formulation containing higher percentage of surfactant, so it is necessary to evaluate the toxicity of formulations.

8.2. Experimental procedure:

8.2.1. Animals:

Albino male rats weighing 200-250gms were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

8.2.2. Nephro-toxicity study of acyclovir and its microemulsion & SLN formulations:

The rats were divided into five groups of four animals each. Group I served as control. Group II received acyclovir (20mg/kg, oral) for 15 days. Groups III, IV received microemulsion formulations (ME04 and ME11) and group V received the SLN formulation (Acy-SLN-opt) containing acyclovir 20mg/Kg, oral respectively for 15 days.

After 24 hours of the last dose of acyclovir, blood was collected and serum was separated for estimations of creatinin, urea and blood urea nitrogen (BUN). The values of urea and BUN were determined with kits of Span Diagnostics (India) Pvt. Ltd. India. The values of creatinin were determined by with kits of Recon Diagnostics Pvt. Ltd., India. DAM method was used for the estimation of urea and BUN whereas alkaline picrate method was used for the estimation of creatinin. The animals were then sacrificed and the kidney was examined macroscopically and cross sections of kidney were collected in 5% formalin in saline for histopathological evaluation. The tissues collected in formalin at necropsy were processed, embedded in paraffin, sectioned at 5microns and stained with hematoxylin and eosin. Histopathologic examination of the tissue sections were conducted by a pathologist and peer reviewed.

8.2.3. *Hepato-toxicity study of efavirenz and its microemulsion & SLN formulations:*

The rats were divided into five groups of three animals each. Group I served as control. Group II received efavirenz as commercially available capsule formulation (20mg/kg, oral) for 15 days. Groups III, IV received microemulsion formulations (ME13 and ME23) and group V received the SLN formulation (Efa-SLN-opt) containing efavirenz 20mg/Kg, oral respectively for 15 days. After 24 hours of the last dose of efavirenz, blood was collected and serum was separated for estimations of SGPT and SGOT. The animals were then sacrificed and the liver was examined macroscopically and cross sections of liver were collected in 5% formalin in saline for histopathological evaluation. The tissues collected in formalin at necropsy were processed, embedded in paraffin, sectioned at 5microns and stained with hematoxylin and eosin. Peer reviewed was made for histopathologic examination of the tissue sections by the help of a pathologist.

8.3. Results and discussion:

8.3.1. Nephro-toxicity studies:

Administration of acyclovir (group II) resulted in a significant elevation in serum creatinin, urea and BUN levels, the markers for renal injury, as compared to control group (Group I). The administration of microemulsion formulation (ME04 and ME11) and SLN (Acy-SLN-opt) showed does not increase in these levels (Table 8.1) The

nephro-toxicity of acyclovir, characterized by the elevation of serum creatinin, urea, uric acid and BUN, was also reversed to a significant extent by the microemulsion and SLN formulation containing acyclovir (ME04, ME11, Acy-SLN-opt). All the results were statiscally analyzed and does not found significant different between two groups. Rat dosed orally with acyclovir (20mg/Kg/day) exhibited no drug related histopathology in kidney at 24hour. However, in the 15^{th} day acyclovir-treated kidneys, necrosis occurred in the tubular epithelial cells located in the outer stripe of the outer medulla (Figure 8.1(b)), necrosis was also accompanied by scattered apoptosis, as evidenced by diminished cells when compared to the control rat kidney (Figure 8.1(a)). Microemulsion and SLN treatment for 15 days had shown comparatively less necrosis on kidney than free drug as shown in Figure 8.2 to 8.3

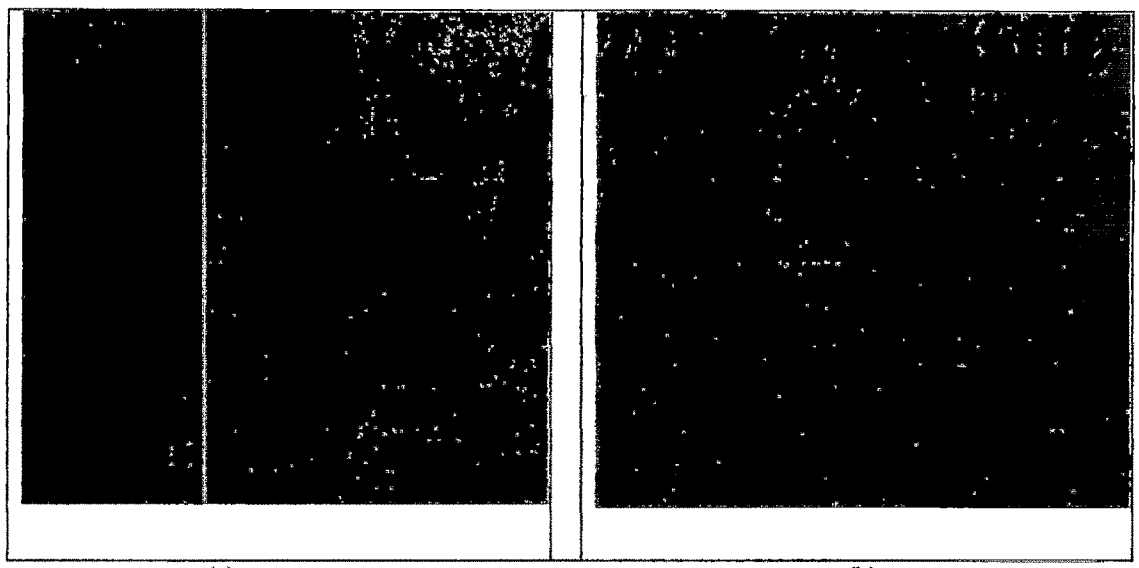
Table 8.1: Nephro-toxicity study of acyclovir and its formulations (microemulsions and SLN) on the serum levels of creatinin, urea, uric acid and BUN.

Groups	Creatinin (mg/dl)	Urea (mg/100ml)	BUN (mg/100ml)
Group I (Control)	0.756	21.61	10.09
Group II (Acyclovir)	0.844	26.69	12.46
Group III (ME04)	0.983	22.11	10.32
Group IV (ME11)	1.008	24.40	11.39
Group V (Acy-SLN-opt)	0.857	23.38	10.92

$P < 0.001$, extremely significant.

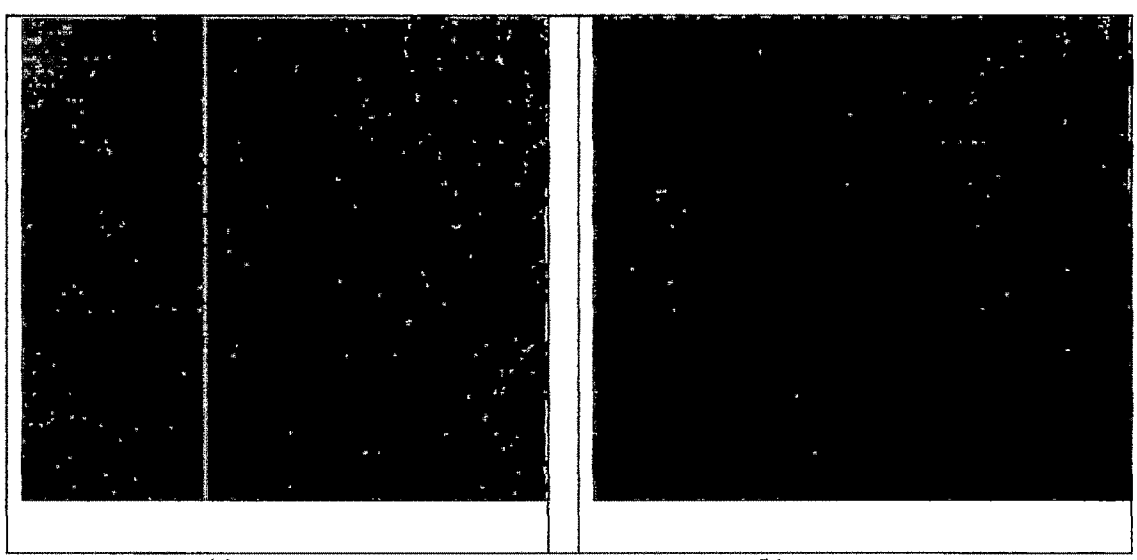
Comparison of groups: Group I and Group II, Group II and Group III, Group II and Group IV, Group II and Group V.

Light micrographs (40X) comparing kidney from control male rats with kidney from male rats treated with acyclovir and formulations (microemulsion and SLN)



(a) (b)

Figure 8.1: Kidney from (a) control male rats showing normal morphology of epithelial cells located in the outer stripe of the outer media (b) rats treated with 20mg/kg/day acyclovir for 15days



(a) (b)

Figure 8.2: Kidney from male rat treated with 20mg/kg/day acyclovir in (a) microemulsion (ME04) (b) microemulsion (ME11) for 15days.

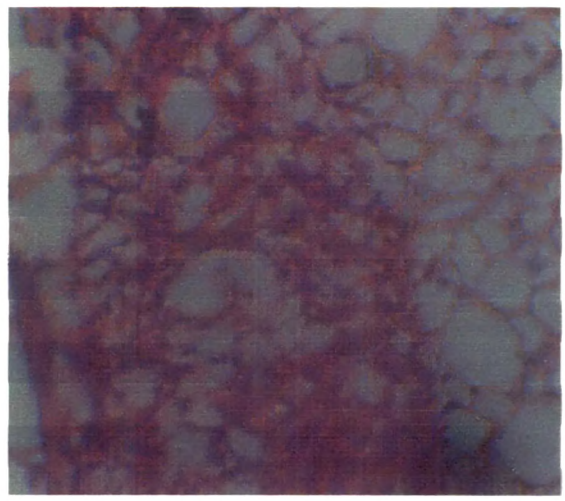


Figure 8.3: Kidney from male rat treated with 20mg/kg/day acyclovir in SLN (Acy-SLN-opt) for 15days

8.3.2. Hepato-toxicity studies:

SGPT is the primary Krebs cycle expressant, occurring as and result of the release of catabolic fats. The SGPT enzyme exists in the serum as consequences of substances being release by the fatty membranes of the liver sinusoids and the lymphatic ducts. The liver products store foods, and the lymphatic ducts store toxins. High SGPT indicates that the primary surfactant (vitamin A) is not sufficient to hold the nutrient and toxic substances. This means there are free-floating sugars, proteins and toxins. The possible reason for low SGPT an excess of membrane-stored nutrients and toxins is not allowing water exchange or fatty congestion of the liver and lymphatics.

SGOT indicates the presence of oxalic acid, a by-product of the conversion of the basic cholesterol molecules into estrogen, progesterone and testosterone. The epithelial cells in the gonads are specific for the conversion, at the level of the mitochondria, of cholesterol into sex hormones. High SGOT indicates excessive oxidation at the level of the membrane and a consequent deficiency of hormones. Low SGOT indicates the glandular function has been impaired by a lack of the conversive epithelium necessary. Table 8.2

indicates the different SGPT and SGOT found at control and treated rat. All the formulation did not elevate the level of SGPT or SGOT

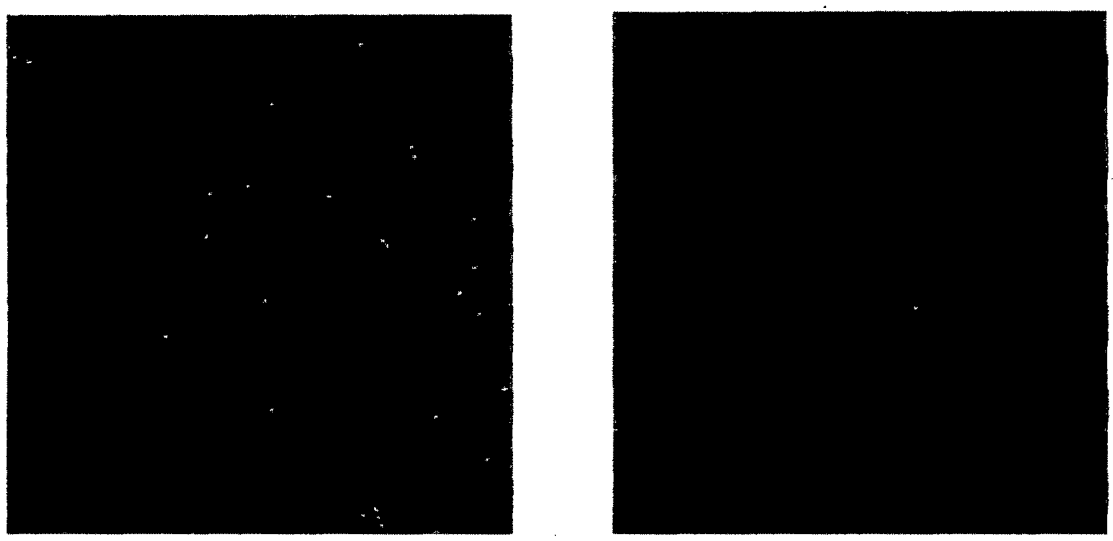
Table 8.2: Hepato-toxicity study of efavirenz and its formulations (microemulsions and SLN) on the serum levels of SGPT and SGOT.

Groups	SGPT	SGOT
Group I (Control)	11.4 ± 1.3	12.3 ± 2.4
Group II (Efavirenz)	16.1 ± 2.1	17.4 ± 1.8
Group III (ME13)	19.6 ± 1.6	21.6 ± 1.4
Group IV (ME23)	18.9 ± 1.8	19.8 ± 0.9
Group V (Efa-SLN-opt)	15.3 ± 1.5	17.5 ± 1.4

$P < 0.001$, extremely significant.

Comparison of groups: Group I and Group II, Group II and Group III, Group II and Group IV, Group II and Group V.

After 15th day treatment with efavirenz (20mg/Kg/day), visibility of hepatic globules with accessories structure such as bile caneli-culi, peripheral and pericentral region in the liver was checked. From the hispathtology studies, it was clear that the microemulsion or SLN formulation does not show any significant damage irrespective to the formulation and SLN formulation are even less toxic than microemulsion formulations. Active drug and microemulsions formulation shows a similar toxic effect. The hepatic globules with accessories structure such as bile caneli-culi, peripheral and pericentral region clearly visible in SLN formulations, whereas for microemulsions as well as for capsule formulations, they are not clearly visible (Figure 8.4 to Figure 8.6)



(a) (b)

Figure 8.4: Liver from (a) control male rats showing normal morphology (b) treated with 20mg/kg/day efavirenz for 15days

(a) (b)

Figure 8.5: Liver from male rat treated with 20mg/kg/day efavirenz in (a) microemulsion (ME13) (b) microemulsion (ME23) for 15days

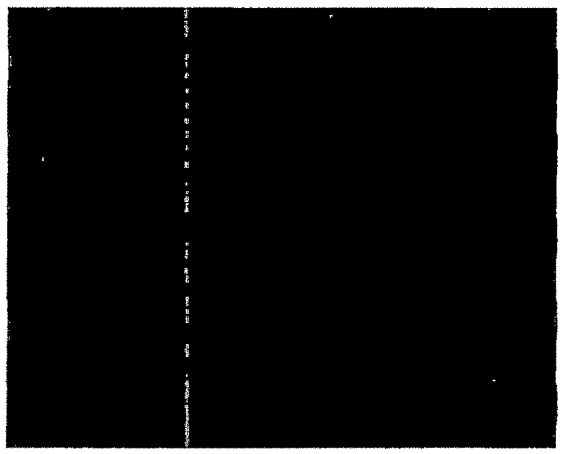


Figure 8.6: Liver from male rat treated with 20mg/kg/day efavirenz in SLN (Efa-SLN-opt) for 15days

Reference:

O'Brien JJ, Campoli- Richards DM., Drugs 1998; 37: 233.