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Acute and Subacute toxicity studies on Valacyclovir and GancicyclovirNiosomal Formulations.

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Abstract

Acute and sub-chronic investigations were used to evaluate the toxicity of niosomal preparations. Rats, both male and female, received optimised formulations in single dose acute trial (F1 & F2) and were tracked for 7 days. The least lethal dose for rats when administered orally was determined because no deaths were noted. Subchronic testing with improved formulations (F1 & F2) (oral gavage) was performed on male and female Wistar rats for 14 days, it found no adverse effects on diet, weight gain, mortality, laboratory tests, pathology, or histopathology. While modest differences in body weight increase (%) and a few haematological markers were seen among treated and control animals, these alterations were mainly of a minor nature and are not thought to have any toxicological importance. With both formulations, no-observable-adverse-effects level was established. In conclusion, these safety studies' findings confirm that both formulations are safe.

Introduction

Niosomes are the vesicles of non-ionic surfactants that are generated when non-ionic surfactants (synthetic) are hydrated, bereft the incorporation of lipids. Vesicle we know today was designed via researcher Handjani-Vila et al.[1]. It is vesicular structures that resemble liposomes and transport lipophilic, hydrophilic, and amphiphilic medications. One justification for the creation of niosomes is that the surfactants are thought to have more chemical stability than the phospholipids utilised in creation of liposomes. Phospholipids have ester bonds, making them very susceptible to hydrolysis[2]. Further difficulties arise from inability to replicate lecithins in liposomes, which is what led one researcher to search for vesicles formed from non-ionic surfactants and other materials. Effective and non-ionic niosomal drug delivery reduces toxicity and improves therapeutic index by focusing drug effects on specific cells. Niosomes, which are microscopic lamellar structures, are created when non-ionic surfactants from the alkyl or dialkyl polyglycerol ether family are combined with cholesterol.[3-4]An example of a non-ionic surfactant that can form amphiphile in niosomes is span 60. Stabilising these vesicles often entails the use of cholesterol and trace amounts of anionic surfactants like dicetyl phosphate. Niosomes gain growing scientific attention in nanotechnology because they are exceptional in their versatility as valuable drug delivery mechanisms for a range of therapeutic applications.

Niosomes are the ideal replacement for non-ionic surfactant-based liposome vesicular nanocarriers. Both liposomes and niosomes are amphiphilic carriers with the same pharmacological uses, physicochemical characteristics, and in vivo behavior[1].Despite having some similar traits, the bilayer's chemical structure is different from that of niosomes, giving it several advantages over liposomes. Liposomes require phospholipids for their formation, while niosomes rely on surfactants with enhanced chemical and biological stability. Additionally, by altering the composition of niosomes in bilayers, enhanced drug trapping is possible. Additionally, because of their higher stability, their industrial processing is less

expensive. Most publications are based on niosome products, emphasizing their optimum capacity for skin permeation, persistent release characteristics, long life, and a strong level of photo-protective function relative to liposome products[5-6].

Materials and methods

Preparation of Niosomal Formulations

Table 1: Composition of Valacyclovir Niosomes formulation Box Behnken Design

F.No	Valacyclovir (mg)	Cholesterol (%)	Brij 72 (%)	Diacetyl Phosphate (%)	Chloroform (ml)	Distilled Water (mL)
F1	500	50	40	6	10	Q.S

Table 2: Composition of Ganciclovir Niosomes formulation by Box Behnken Design

F.No	Ganciclovir (mg)	Cholesterol (%)	Span 60 (%)	Diacetyl Phosphate (%)	Chloroform (ml)	Distilled Water (mL)
F2	250	50	30	6	10	Q.S

Experimental animals

Male (8-12 weeks old, 150-200 g body weight) and female (120-150 g body weight) Wistar albino rats were used to test for acute and sub-acute toxicity. Each polypropylene cage held three rats. Both rats were placed separately, and chosen females were not carrying any babies. Prior to testing, animals were housed in sterile polypropylene cages for week in animal room. Cages were maintained under typical circumstances (65% relative humidity; 25± 2 °C; 12/12 hr light/dark cycle). The bedding made of paddy husk. The rat pellets were animals given as is customary (M/s Hindustan Lever Ltd., Mumbai), along with water. Srikrupa Institute of

Pharmaceutical Sciences' IAEC (Institutional Animal Ethics Committee) in Narsampet, Warangal, Telangana, India (CPCSEA Number: 03/SKIPSc/IAEC/2018) authorised all experimental procedures. Ethics guidelines for the care and use of laboratory animals as outlined in the Guide for the Care and Use of Laboratory Animals have been strictly adhered to.

LD_{50} study

Acute toxicity studies used Wistar albino rats and were carried out mostly in accordance with OECD standards 423, with a few modifications. Acute toxicity experiments were performed on Wistar albino rats and were carried out mostly in accordance with OECD rules 423, with a few minor modifications. The investigation was conducted using male and female rats. After that, we divided the chosen males and females into two groups of five for the standard control and treatment rats. Test sample was given orally to test group of rats at dose of 2000 mg/kg, b.wt. Rats in control group were only given water to drink. The rats were all weighed, given a body mark to identify them, and starved the night before the experiment. They did, however, have unrestricted access to water. Following dosing, the rats continued to fast for the next four hours while being continuously observed for each individual. Then, they were watched twice a day for a week to see if they had undesirable effects, like changes in the colour of their eyes, hair, and skin, as well as changes in how much they ate and drank, as well as any shaking salivation, , convulsions, lethargy, diarrhoea, breathing, strange behaviour, sleep, motor activity, or coma. Because of this, the test extract's acute toxicity level was checked to help choose doses for repeated oral toxicity experiment.

Sub-acute toxicity studies

White albino the sub-acute toxicity of niosomal preparations (F1 and F2) was studied using Wistar rats following the steps outlined in OECD guideline 407. **Body weight Variation**

Each rat's body weight was measured starting on Day 0 (the first day of treatment), then every week after that for the duration of the study. One day exactly before blood was taken and the animals were kept to fast overnight (Day 15), the final body weight was noted.

Organ Weight

The 42 animals' organs that were gathered and weighed .Organs were removed, cleaned using Analytical balance (Made: Mettler Toledo) weighted with filter paper. The weights of every organ were record as absolute values.

Evaluation of body weight and organ weight

Researchers compared the weights of the untreated and treated rats to look for signs of toxicity. A macroscopic examination of the target organs was performed on two formulations (F1, F2) to check for any weight, texture, or shape anomalies that would be dangerous. One of the main organs targeted is the rat's thymus, along with the kidneys, liver, lungs, and spleen.

Hematological analysis

Blood was collected from Wistar rats under general anaesthesia and then tested for haematological factors such RBC, WBC, Hb and platelet count in capillary tubes. [28,29].

Biochemical analysis

Blood was centrifuged for ten minutes at a speed of 3000 g at for biochemical analysis, 4 °C. After centrifuging the blood, the serum was collected and frozen at -20 degrees Celsius for later use. Standardised procedures were used to analyse biochemical parameters such as alanine transaminase (ALT), urea, albumin, aspartate transaminase (AST), alkaline phosphatase (ALP), serum creatinine, and total protein.

Histopathological studies

On organ samples from the liver, pancreas, and kidney, histopathological investigations were carried out. All animals were put to sleep, and after autopsies, the major organs, such as Surgery was used to remove the stomach, small intestine, liver, and kidney, which were then fixed in 20% formalin in normal saline. Hematoxylin-eosin (HE) 40 was used to stain the sample after 5 m sections were cut on a rotary microtome. Following that, the sections underwent a microscopic analysis for pathological exams.

Results and discussion

The acute toxicity investigations did not reveal any fatalities. 4 hours after the start of the observation period and 24 hours later. Additionally, following administering the niosomal formulations for the seven-day trial period, no deadly effect was seen. Fur, eyes, skin, and nose morphological features appeared ordinary. No unusual behaviours were seen, such as excessive salivation, diarrhoea, or lethargy. The patient's breathing, body weight, food and drink intake, and overall health were all normal. However, 10 to 15 minutes after bothniosomal preparations were given, significant sedation was observed. Niosomal formulations were not associated with any known deaths.

Subacute toxicity study

Body weight Variation

On Day 14, the final day of the experiment, every single one of the 5 male and female rats was still alive and appeared normal. Each animal's body weight grew during the trial. **Organ Weight**

At end of treatment period, neither males nor females had different organ weights.**(Table 3).**

Table 3: Effect of niosomal formulations on organ weights

Formulations	Liver	Kidneys	Lungs	Thymus
F1	2.44±0.31	0.52±0.05	0.35 ± 0.04	0.07 ± 0.01

F2	2.32±0.22	0.54±0.04	0.34 ± 0.04	0.08 ± 0.01

Haematological analysis

No changes with toxicological significance were discovered during the 14 days of therapy. Assessments of the haematological data. Some treatment groups showed statistically significant changes on some measures when compared to control group. Lack of a dose-response relationship and absence in females led researchers to conclude that these variations were of little biological significance. No statistically noteworthy changes were seen in any of urinalysis or blood biochemistry measurements. Haematological and serum biochemical indicators showed no notable alterations at the conclusion of the recovery period. There were no obvious treatment-related lesions found after necropsy. No organ alterations occurred. weight either for male or females, at conclusion of therapeutic term. The results were not thought to be biologically significant because the liver weights of the control females were lower, the high-dose males did not show a similar effect, there were no histopathological correlates in the liver, and there were no effects on serum biochemistry and blood parameter results, which are used to measure liver function **(Table 4).**

Table 4: Niosomal formulations effect on hematological parameters

Formulations	Hb (g/dl)	RBC (T/L) (Tera = 1 trillion)	WBC (G/L)	Platelets (S)
F1	15.9 ± 0.4	9.33 ± 0.41	5.1 ± 1.4	16.3 ± 1.4
F2	15.7 ± 0.7	9.47 ± 0.48	4.5 ± 1.2	17.6 ± 1.1

Selected hematology values for n=6 Values are mean ± standard deviation

Biochemical analysis

EIn Wistar rats, the effect of daily oral administration of niosomal formulations over a period of 14 days was assessed. Neither blood biochemistry analyses nor urinalysis measurements

showed any statistically significant differences. There were no noticeable changes in haematological and serum biochemistry indicators were found at conclusion of recovery phase. Biochemical parameters were measured for each group and compared to healthy control; results are presented in Table 5.

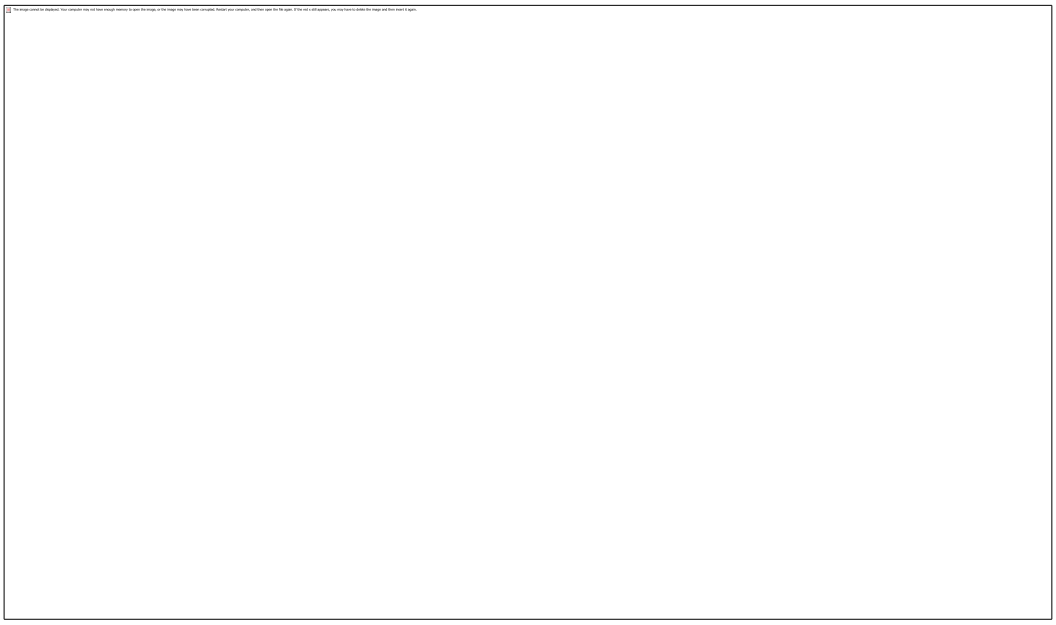
Table 5: Effect of niosomalformulations on biochemical parameters

Formulations	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	UREA (mg/dl)	CRE (mg/dl)	TP (mg/dl)	ALB (g/l)
F1	77.4±10.3	35.5±8.2	56.4±10.8	5.17±0.90	27.6± 2.8	68 ± 2	44 ± 2
F2	69.8 ± 7.5	32.7±5.3	62.6 ± 8.8	4.78±0.82	27.5± 2.4	73 ± 4	43 ± 3

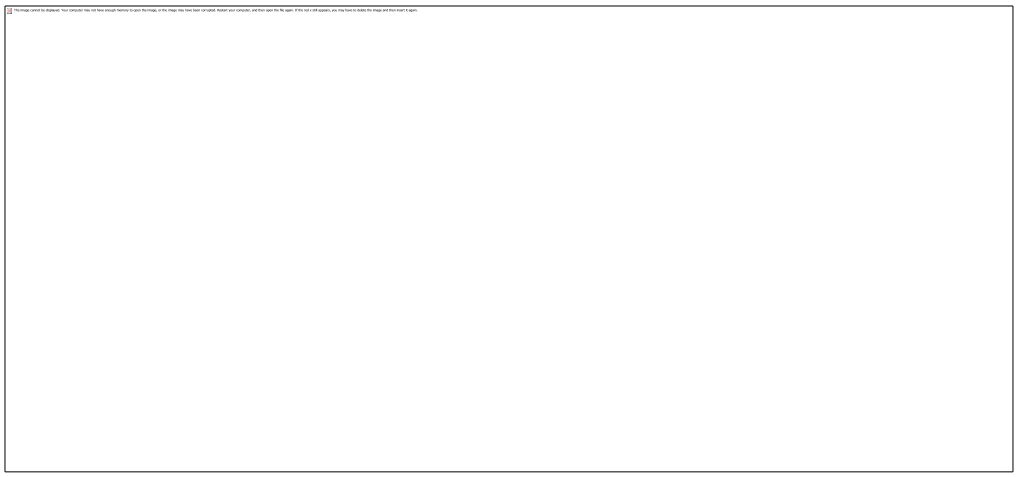
Histopathological studies

Finally, histological examination revealed no clearly treatment-related side effects. High-dose females were more likely to experience inflammatory infiltration of the stomach. All animals in each group had their livers, kidneys, stomachs, and small intestines collected for histological study during necropsy.

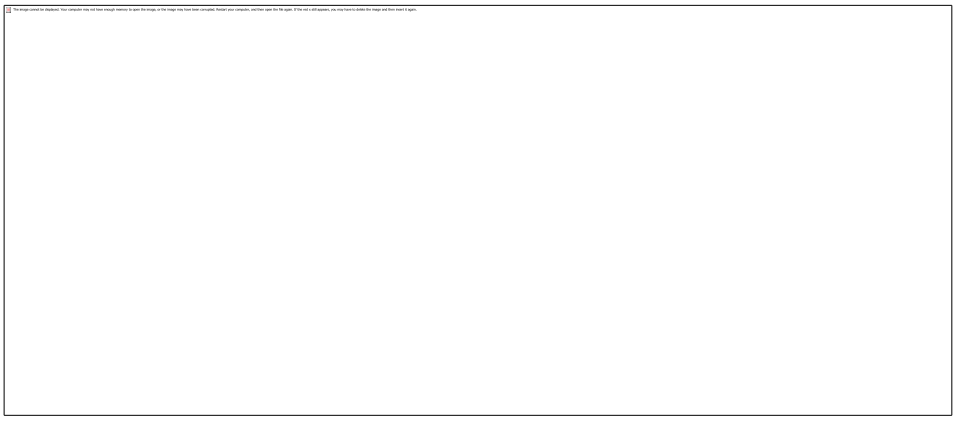
Stomach: Surface necrosis and mucosal epithelial degradation were visible in a portion of the stomach. Some of the animals' serosal stomach surfaces were thicker and fibrinous exudates were deposited there. Neutrophil infiltration was clearly seen in exudates on the serosal surface.



Kidney: A small amount of tubular parenchymatous deterioration was visible in a kidney slice. While there were no evident changes in the glomeruli.



Liver: There were no liver histological alterations compared to the control animals. However, livers of a few of the animals showed fatty changes.



Conclusion

Acute toxicity is regarded as a preliminary investigation that gives us the foundation for categorization and labelling. It also offers preliminary data on the mechanism of toxicity of a substance, allowing us to set a dose of novel compound and aid in dose estimation in animal investigations. Preliminary markers of early signs of toxicity induced by numerous substances and medicines include general behavioural changes and body weight. No rats died after subacute niosomal formulations consumption, and both male and female rats' behaviour alterations were hardly noticeable. In conclusion, the information presented here shows that both niosomal formulations are well tolerated, together with data on human efficacy. Both the produced niosomal formulations of Valacyclovir and Gancicyclovir are therapeutically important and had no impact on changes in body and organ weight, haematological parameters, or biochemical parameters. Nearly all of the parameters were normal and showed no significant changes, although a few parameters showed modest changes that may or may not have been caused by the medication and hence had little or no toxicological significance. However, more research is need to validate its efficacy and safety in humans.

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