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Formulation And *In Vivo* Evaluation Of Ritonavir Nanosuspension

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ABSTRACT

Aim: Formulation and *in vivo* evaluation of Ritonavir nanosuspension.
Objectives: To study the significance variance in the pharmacokinetic parameters of ritonavir.
Methods: Blood samples were collected and studied for Pharmacokinetic parameters.
Results: It was observed that when compared to pure drug, nanosuspension had improved pharmacokinetic properties.
Conclusion: In the current study, the prepared NS have better bioavailability when compared to pure drug.

Key Words: Ritonavir, Nanosuspension, Pharmacokinetic parameters.

1.0 INTRODUCTION

Ritonavir is a medication in the anti-retroviral class. It serves as an inhibitor of proteases. Protease inhibitors prevent the function of protease, an enzyme that is necessary for the virus's ultimate development[1–5]. Protease inhibitors stop the synthesis of infectious viral particles by inhibiting the activity of proteases. Ritonavir is a medication that inhibits the HIV viral proteinase enzyme, preventing the cleavage of the gag-pol polyprotein and the subsequent production of immature, non-infectious viral particles. HIV's protease enzyme is blocked by protease inhibitors. The proteolytic cleavage of the viral polyprotein precursors into the distinct functional proteins present in infectious HIV-16-9 is accomplished by HIV-1 protease, an enzyme that is necessary. The drug suppresses the activity of the enzyme by binding to the active site of the protease. This inhibition stops the viral polyproteins from cleaving, which would otherwise produce immature, non-infectious viral particles. Protease inhibitors are nearly always used in conjunction with two or more anti-HIV medications.

2.0 METHODS

PHARMACOKINETIC EVALUATION OF OPTIMIZED NANOSUSPENSIONS OF CLASS IV DRUG RITONAVIR

Preparation of Ritonavir solution:
20 mg of Ritonavir was dissolved in 10 ml of 0.1 N HCl, and the mixture was then filtered through 0.22 μm sterile graded filters in a sterile room while maintaining aseptic conditions, all into a glass vial[10].

Preparation of Ritonavir nanosuspensions
Male Wistar rats were used in experiments with a subset of the study's products. Ritonavir's tailored nanosuspensions were reconstituted in 10 milliliters of pH 1.2 Buffer containing 0.1 N HCl for the investigation. The reconstituted nano dispersions were found to disperse easily and to provide a homogeneous dispersion after reconstitution.

Animals:
The male Wistar rats used in the in vivo experiments weighed between 200 and 250 g. The Institutional Animal Ethics Committee, 1219/PO/Re/S/08/CCSEA, examined and approved each study. Following the purchase of rats, they were allowed to acclimate to room temperature (25±3ºC) for at least one week. Prior to research, the animals were fasted for one night, although they were allowed unlimited access to water during the investigation.

Study design
Ritonavir
The animals were split up into two groups, with six animals in each group. Group 2 received optimized nano suspensions whereas Group 1 received pure ritonavir solution. For the current investigation, the animal dose for rats was determined to be 10 mg/kg. Oral gavage of 1 ml containing the necessary dose was performed at a rate of 0.4 ml/min.

Blood sampling:
The rat's retro orbital sinus was used to draw blood samples (3 µl aliquots for each drug) which were then placed in micro centrifuge tubes containing dipotassium ethylene diamine tetraacetic acid. Samples were drawn at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours after dosing, and the plasma was separated immediately after by ultracentrifugation at 2–8°C for 15 minutes at 5000 rpm. Each plasma sample was then collected and stored at –20°C until the drug was analyzed.

Determination of pharmacokinetic parameters
Using non-compartmental analysis, the pharmacokinetic characteristics of five formulations were determined from each individual plasma drug concentration-time profile. Using the data, the following pharmacokinetic characteristics were determined for each case: biological half life (t½), elimination rate constant (Kel), and area under the curve (AUC0-t). The Kinetica 4.4.1 software trial version (Thermo Fisher Scientifics Inc. CA, USA) was used for all pharmacokinetic calculations.

Peak plasma concentration (C_{max})
The highest plasma drug concentration that can be attained following oral medication administration is known as the peak plasma concentration. C_{max} indicates if the medication is systemically absorbed enough to produce a therapeutic effect.

Time of peak plasma concentration (T_{max})
The amount of time needed to achieve the maximum drug concentration following drug delivery is equal to the peak plasma concentration. Drug absorption reaches its peak at Tmax, when the rate of absorption precisely matches the rate of drug excretion.

Area under plasma concentration-time curve (AUC)
The area under the plasma level-time curve can be used to calculate the bioavailability of a medicine. The AUC represents the total quantity of active medicine that enters the systemic circulation. The area under the drug plasma level-time curve (AUC) from t=0 to t=∞ is obtained by dividing the amount of unchanged drug that reaches the general circulation by the clearance.
By adding the AUC0-t and the extrapolated area under the curve to time infinity (AUCt-∞), which was equal to the ratio of Ct/Kel, one can determine AUC0-∞, the area under the plasma drug concentration-time curve from time zero to infinity.

Apparent terminal elimination rate constant (K_{el})
The log plasma concentration vs. time curve was used to calculate the apparent terminal elimination rate constant. The linear section of the elimination phase has a slope of Kel.

3.0 RESULTS AND DISCUSSION

The treatment of drug by the healthy rat subjects as they did not show of any adverse effects during the course of the study. There were no signs of gastric tract disturbances or allergic reactions were observed in any of the subjects during the study. Ritonavir plasma concentrations after single oral administration of the two treatments in the individual rats are shown in Tables 1, 2 and the respective plasma concentration-time profiles are given in Figs 1, 2 and 3. Pharmacokinetic data of Pure Ritonavir and Ritonavir nanosuspensions were shown in tables 3 and 4.

Table 1: Plasma concentration profiles of Ritonavir pure drug at different time intervals

	Plasma concentration (ng/ml) of Ritonavir												
Rat	**Time (hrs)**												
	0.5	1	2	4	6	8	10	12	14	16	18	20	24
1	33.5	185.3	285.7	192.6	143.9	101.7	90.2	75.3	51.7	45.7	39.3	26.5	2.2
2	37.5	176.1	276.9	191.7	141.6	104.6	89.9	77.5	53.5	43.5	38.7	28.3	2.5
3	38.9	152	252.3	193.5	145.7	105.2	90.3	76.3	50.7	44.9	36.3	29.5	3.7
4	40.1	160	260.5	199.6	142.5	103.3	92.5	75.4	52.5	45.1	38.1	27.9	4.1
5	41.3	176	276.3	195.3	149.9	108.9	95.2	76.9	51.9	46.6	37.3	25.6	4.5
6	40.9	170	270.5	195.2	141.3	101.7	91.7	77.8	55.6	43.9	34.6	24.3	4.9
Mean	38.7	169.9	270.3	194.6	144.4	104.2	91.6	76.5	52.6	44.9	37.3	27	3.65
S.D	0.6	1.2	0.5	1.1	0.7	0.9	1.2	1.5	1.1	1.2	0.6	0.2	0.3

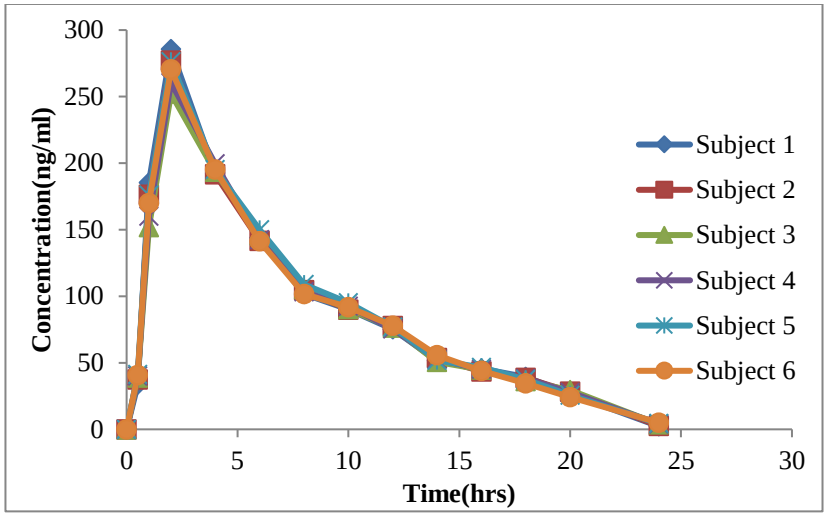

Fig 1: Plasma concentration profiles of Ritonavir pure drug at different time intervals

Table 2: Plasma concentration profiles of Ritonavir nanosuspension at different time intervals

	Plasma concentration (ng/ml) of Ritonavir nanosuspension												
Rat	**Time (hrs)**												
	0.5	1	2	4	6	8	10	12	14	16	18	20	24
1	199.3	423.2	376.2	299.3	244.3	199.3	177.5	122.3	93.7	61.9	33.5	19.2	4.2
2	198.7	436.9	377.5	289.1	239.5	195.6	176.3	125.8	95.6	63.5	37.9	18.6	4.6
3	186.3	442.7	372.8	295.2	241.3	190.2	175.9	125.2	96.5	65.8	36.5	18.7	4.8
4	185.1	459.3	379.4	291.3	239.5	194.3	174.8	127.6	97.6	66.9	38.1	18.5	4.1
5	189.2	441.8	377.2	293.6	241.5	192.9	178.3	128.5	98.2	66.7	33.2	15.6	4.7
6	199.2	438.1	376.1	294.7	244.6	189.5	176.7	133.5	96.1	67.5	32.9	17.3	4.5
Mean	192.9	440.3	376.5	293.8	241.7	193.6	176.5	127.1	96.2	65.3	35.3	17.9	4.4
S.D	3.2	3.2	2.2	2.3	3.3	0.6	1.2	1.5	2	1.2	0.6	0.2	0.2

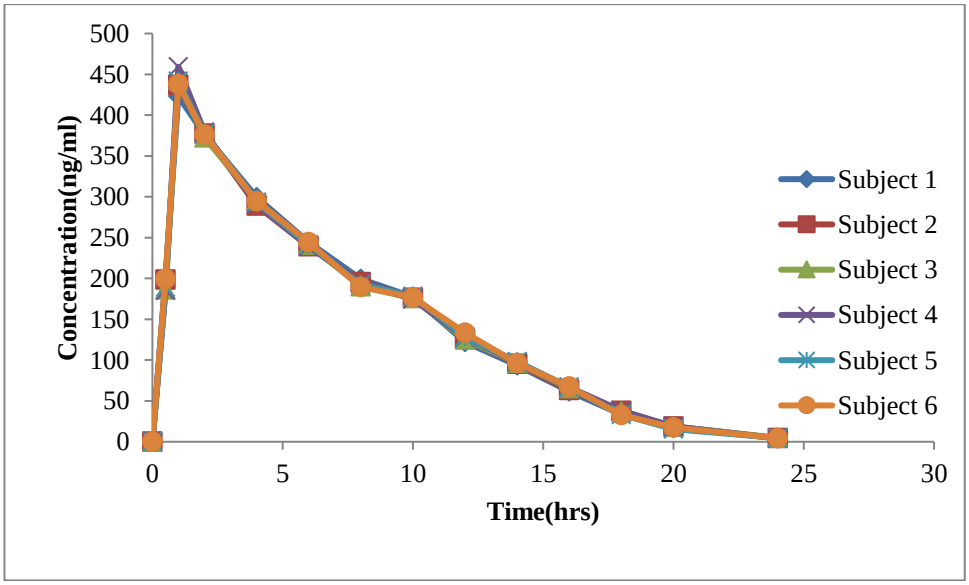

Fig 2: Plasma concentration profiles of Ritonavir nanosuspension at different time intervals

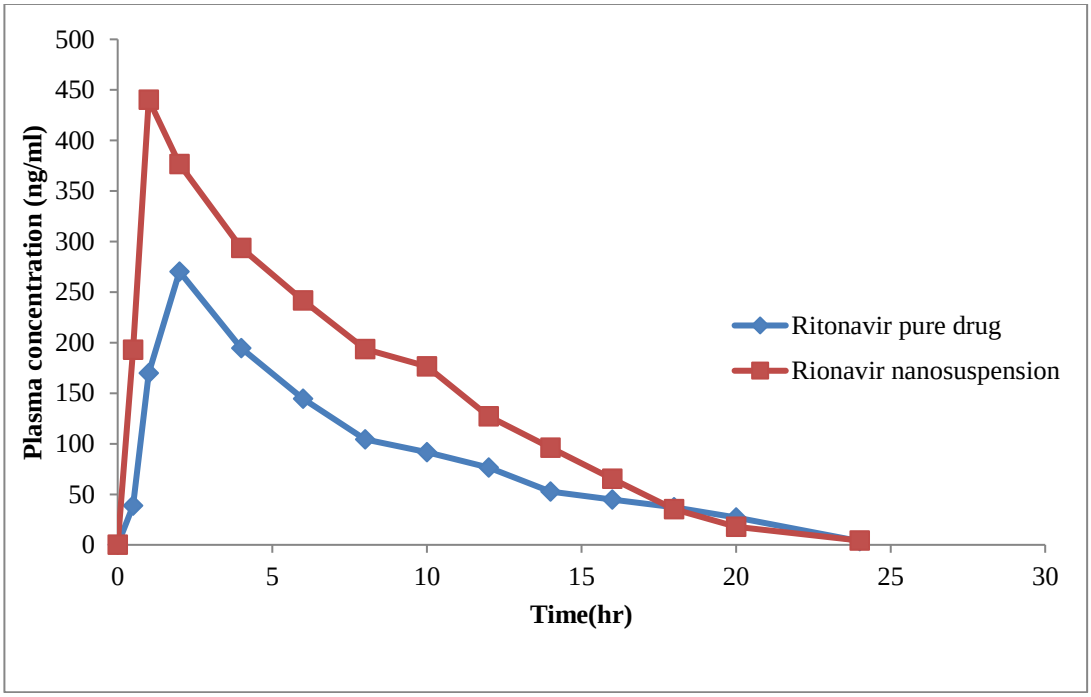

Fig 3: Comparison graph between pure Ritonavir and optimized formulation

Table 3: Pharmacokinetic data of Pure Ritonavir

Treatment	Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (h*ng/ml)	$AUC_{0-\infty}$ (h*ng/ml)	K_{el}	$t_{1/2}$ (h)
Reference	1	2.0	285.7	1103.5	1320.5	0.11	6.3
	2	2.0	276.9	1109.6	1359.7	0.106	6.5
	3	2.0	252.3	1214.9	1432.9	0.111	6.2
	4	2.0	260.5	1113.5	1532.7	0.113	6.1
	5	2.0	276.3	1109.7	1499.3	0.11	6.3
	6	2.0	270.5	1135.6	1530.1	0.111	6.2
	N	6	6	6	6	6	6
	Mean	2.0	257	1131.1	1445.8	0.110	6.26
	SD	0.9	11.9	11.9	25.3	2.3	5.5

Table 4: Pharmacokinetic data of Ritonavir nanosuspension

Treatment	Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (h*ng/ml)	$AUC_{0-\infty}$ (h*ng/ml)	K_{el}	$t_{1/2}$ (h)
Reference	1	1.0	423.2	1425.3	1632.5	0.100	6.9
	2	1.0	436.9	1527.9	1649.3	0.097	7.1
	3	1.0	442.7	1545.8	1658.9	0.099	7.0
	4	1.0	459.3	1528.3	1633.2	0.100	6.9
	5	1.0	441.8	1548.1	1674.1	0.099	7.0
	6	1.0	438.1	1547.7	1679.1	0.100	6.9
	N	6	6	6	6	6	6
	Mean	1.0	440.3	1520.1	1654.3	0.099	6.96
	SD	0.9	2.3	1.9	5.3	0.9	2.4

4.0 Conclusion

Tables 1-2 display the plots of the mean plasma concentration of the Ritonavir in both the test and reference, and Figs 1-2 compare the plasma profiles of the Ritonavir nanosuspension (T) and pure medication (R). Tables 3 and 4 provided an overview of the pharmacokinetic characteristics. The Cmax, Tmax, and AUC of Ritonavir nanosuspension were

significantly higher than those of the pure medication. The pure medication had a lower Cmax than the nanosuspension. Furthermore, pure Ritonavir was shown to have a greater AUC0-t of 1131.1 h *ng/ml than the nanosuspension, at 1520.1 h ng/ml. Similarly, pure Ritonavir was shown to have a greater AUC0-∞ of 1445.1 h*ng/ml than the nanosuspension, which had an AUC0-∞ of 1654.2 h*ng/ml. Still, the tmax value

5.0 References

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