9. In-vivo Characterization

9.1. Introduction

With the aim of addressing poor bioavailability(1, 2) and gastrointestinal related side effects(3, 4) associated with currently available marketed formulations of both TAC and FBX, the most preferred alternative to oral route i.e. transdermal route has been explored. As described in previous chapters, cubosome loaded fast dissolving MN patches of both drugs were successfully developed and evaluated in vitro as well as ex vivo for characteristics, most suited for transdermal delivery. Present chapter has been devoted to pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of TAC and FBX via their newly developed formulations. The in vivo studies were performed to provide a better insight regarding the potential of these newly developed formulations in solving the aforesaid problem.

9.2. Materials and Methods

9.2.1. Materials

Potassium oxonate and CFA (complete Freund's adjuvant) was purchased from TCI, India and Sigma-Aldrich India, respectively. Oral tablet of Tacroflast (TAC) and Febutex (FBX) was purchased from local medical store. Various formulations used for study were developed as described in previous chapter 5, 6 & 7.

9.2.2. Animal study protocol approval

Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India guidelines were used for performing the in-vivo studies. Approval was taken from Institutional Animal Ethics Committee of Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, India for the protocols of both pharmacokinetic and pharmacodynamic studies of TAC (No.: MSU/IAEC/2019-20/1902) and FBX (No.: MSU/IAEC/2019-20/1921).

9.2.3. Animal Procurement

For conducting in vivo studies, healthy female Sprague-Dawley (SD) rats having weight of 200-270 g were employed. Rats used in the experiment were received from

Zydus Research Centre located in Ahmedabad. After the rats were received, they were kept in suitable cages in the animal house facility of Faculty of Pharmacy recognized by CPCSEA (Reg. No.: 404/PO/Re/S/01/CPCSEA; dated 28th October, 2015). The temperature of the room where animals were housed was kept at 21-23 °C and a light-dark cycle was set at 12:12 h. Animals were kept under a standard diet and were given free access to water and allowed one-week acclimatization period before initiating the experiments. Good Laboratory Practice was followed for animal handling routines.(5)

9.2.4. Animal dose calculation

TAC and FBX at their lowest recommended human dose (1 and 40 mg/day, respectively) were used for both PK and PD studies. According to the guidelines set by USFDA, animal equivalent dose (AED) of TAC and FBX was calculated using Eq. 9.1.(6)

$$AED = \frac{\left(\frac{D_H}{W_H}\right) x\ K_H}{K_A}$$

Equation- 9.1

where,

AED = animal equivalent dose (in mg/kg);

D_H = Human daily dose (TAC 1 mg; FBX 40 mg);

W_H = average weight of healthy human adult (60 Kg);

K_H = Human constant (37) and

K_A = Animal constant (6)

For the estimation of accurate dose that is to be administered in individual rats, AED which was calculated for TAC (0.3 mg/Kg) and FBX (4.03 mg/Kg) was utilized.

9.3. Pharmacokinetic of Tacrolimus/Febuxostat

Sprague dawley(7, 8) rats weighing 200-270 g, were procured from an official CPCSEA breeder. Rats which were obtained were placed in cages present in the animal house wherein the temperature was set at 22 ± 3 °C and light-dark cycle of fixed 12 hours was maintained. Handling of the animals was done with compliance to CPCSEA guidelines,

Department of Animal Welfare, Government of India. Rats were kept on standard chow diet and were given water as desired. 30 rats were allocated in 5 groups randomly as shown in table 9.1 & 9.2. Each group had two sets and each set had three animals. All group animals were fasted 12 hours before starting the experiment. A suspension of marketed TAC capsule/ FBX tablet in water was prepared and administered to Group 1 animals through the oral route using oral feeding needle attached to syringe. Transdermal patch of TAC/ FBX was applied on group 2 animals on abdominal skin after removing skin hair. Group 3 animals were applied with cubosomal gel of TAC/FBX, MN patch of TAC/FBX was applied on group 4 animals on abdominal skin after removing hair skin. Cubosomes loaded MN patches of TAC/FBX were applied to group 5 animals on abdominal skin after removing skin hair. Diethyl ether was employed as an anesthetic agent for inducing unconsciousness in rats. Retro orbital route was used for the collection of blood samples (Not more than 0.5 ml) and these samples were kept in microcentrifuge tubes containing heparin at 1, 3, 5, 8, 24 hour from set-1 and 2, 4, 6, 12 hour from set-2 resulting 9 time points (1, 2, 3, 4, 5, 6, 8, 12, 24 hour). The rats were replenished with saline solution. These blood samples were exposed to centrifugation wherein the RPM was set at 3500 rpm for a period of 10 min at a temperature of 4°C. and harvested samples of plasma were analyzed as described in chapter 3 to estimate pharmacokinetic like parameters like C_{max}, T_{max}, $T_{1/2}$, AUC, and MRT.(9, 10)

Table 9.1: Animal grouping for pharmacokinetic study of TAC

Sr. No.	Groups		
	Treatment	Set-I	Set-II
1	Marketed oral suspension of TAC (0.3 mg/kg)	3*	3*
2	Transdermal patch of TAC (0.3 mg/kg)	3*	3*
3	Developed cubosomes of TAC (0.3 mg/kg)	3*	3*
4	MN patch of TAC (0.3 mg/kg)	3*	3*
5	Cubosomes of TAC loaded MN patch (0.3 mg/kg)	3*	3*
Total		**30**	

* Not to be sacrificed, rehabilitation will be done. (@$ Animal will be not sacrificed and used in pharmacodynamic study after washing period)

Table 9.2: Animal grouping for pharmacokinetic study of FBX

Sr. No.	Groups		
	Treatment	Set-I	Set-II
1	Marketed oral suspension of FBX (4.07 mg/kg)	3*	3*
2	Transdermal patch of FBX (4.07 mg/kg)	3*	3*
3	Developed cubosomes of FBX(4.07 mg/kg)	3*	3*
4	MN patch of FBX (4.07 mg/kg)	3*	3*
5	Cubosomes of FBX loaded MN patch (4.07 mg/kg)	3*	3*
Total		**30**	

* Not to be sacrificed, rehabilitation will be done. (@$ Animal will be not sacrificed and used in pharmacodynamic study after washing period)

9.4. Pharmacodynamic of Tacrolimus

Sprague dawley(7) weighing 200-270 g, were procured from an official CPCSEA breeder. Rats which were obtained were placed in cages present in the animal house wherein the temperature was set at 22 ± 3 °C and light-dark cycle of fixed 12 hours was maintained. Handling of the animals was done with compliance to CPCSEA guidelines, Department of Animal Welfare, Government of India. Rats were kept on standard chow chart and were given water as desired. 15 rats were allocated in 5 groups randomly as shown in table 9.3. All animals except Normal control (group 1) were sensitized with CFA (complete Freund's adjuvant) (0.1 ml, sub plantar) for induction of RA.(11) Paw volume of rats was estimated in all groups based on volume displacement method using Plethysometer. Group 3 was administered TAC (0.3 mg/kg orally) for 28 days as a standard control and was compared with other group. Group 4 was treated with cubosomes loaded MN patch of TAC for 28 days. After 0 and 28th day, not more than 0.5 ml blood was withdrawn from the retro-orbital plexus route. All animals were euthanized humanely using overdose of diethyl ether for harvesting rat's leg for X-ray as listed in table 9.4.

Table 9.3: Animal grouping for pharmacodynamic study of TAC

Sr. No.	Group	Treatment	No of animals
1	Normal control	Distilled water	3**
2	Model control	CFA (0.1 ml of 10 mg/ml, Sub Plantar)(15)(11)	3**
3	Standard control	TAC (0.3 mg/kg orally.) + CFA (0.1 ml of 10 mg/ml, Sub Plantar)(11)	3**
4	Test control	Cubosomes loaded MN patch of TAC (0.3 mg/kg transdermally) + CFA (0.1 ml of 1 mg/ml, Sub Plantar)(11)	3**

****** To be sacrificed (@$ Animals of Pharmacokinetic study will be used here after washing period)

Table 9.4: Parameters to be investigated for pharmacodynamic study of TAC

Sr. No.	Parameters	Biological Sample	Parameters to be investigated
1	Physiological parameters	-	Body weight, Paw volume measurement
2	Biochemical parameter	Blood	RhF (Rheumatoid Factor)
3	X-Ray	Bone	-

9.4.1. Paw volume determination

Plethysometer was employed for the measurement of paw volume in rats. Paw volume was measured on the initial day i.e. 0^{th} day prior to injecting CFA and readings were continuously taken at regular intervals until the 25^{th} day of experiment. Change in hind paw volume was measured by subtracting final paw volume from initial paw volume.(12)

9.4.2. Body weight determination

Body weight of the animals was recorded. The first reading was taken on 0^{th} and continuous readings were taken through the end of the experiment. Change in body weight was calculated by subtracting final weight from initial weight.(12)

9.4.3. Measurement of Rheumatoid Factor (RhF)

When RA was induced in rat model, there was an increase of Rh factor in rat blood which was measured quantitatively using RF turbilatex kit purchased from Eurodiagnostics, Chennai, Tamil Nadu, India.(13)

9.4.4. X-ray

X-ray of rat's leg was taken at Angela Lobo clinic, Vadodara after harvesting it form rat after euthanizing humanly to study change in bone shape after completion of pharmacodynamic study. X-ray of all animal's paw from all the groups was taken to study efficacy of given treatment.

9.5. Pharmacodynamic of Febuxostat

Sprague dawley(8) weighing 200-270 g, were procured from an official CPCSEA breeder. Rats which were obtained were placed in cages present in the animal house wherein the temperature was set at 22 ± 3 °C and light-dark cycle of fixed 12 hours was maintained. Handling of the animals was done with compliance to CPCSEA guidelines, Department of Animal Welfare, Government of India. Rats were kept on standard chow diet and were given water as desired. 12 rats were allocated in 4 groups randomly as shown in table 9.5. All animals except Normal control (group 1) were sensitized with Potassium oxonate (PO) (250 mg/kg in 0.9% saline solution, intraperitoneally- IP) for induction of hyperuricemia which is responsible for gout.(14, 15) Paw volume of rats were determined in all groups. Group 3 was treated with FBX (4.07 mg/kg orally) for 28 days as a standard control and compare with other group. Group 4 was treated with Cubosomes loaded MN patch of FBX for 28 days. After 0 and 28^{th} day, not more than 0.5 ml blood was withdrawn from retro-orbital plexus route. All animals were euthanized humanely using overdose of diethyl ether for harvesting rat's leg for X-ray as listed in table 9.6.

Table 9.5: Animal grouping for pharmacodynamic study of FBX

Sr. No.	Group	Treatment	No of animals
1	Normal control	Distilled water	3**
2	Model control	PO* (250 mg/kg in 0.9% saline solution, Intraperitoneally-IP)(14, 15)	3**
3	Standard control	FBX (4.07 mg/kg orally) + PO (250 mg/kg in 0.9% saline solution, Intraperitoneally-IP)(14, 15)	3**
4	Test control I	Cubosomes loaded MN patch of FBX (4.07 mg/kg transdermally) + PO (250 mg/kg in 0.9% saline solution, Intraperitoneally)(14, 15)	3**
Total			**12****

***** PO: Potassium Oxonate

****** To be sacrificed (@$ Animals of Pharmacokinetic study will be used here after washing period)

Table 9.6: Parameters to be investigated for pharmacodynamic study of FBX

Sr. No.	Parameters	Biological Sample	Parameters to be investigated
1	Biochemical parameter	Blood	Uric acid
2	X-Ray	Bone	-

9.5.1. Measurement of Uric Acid (UA)

When gout was induced in rat model, there was an increase in uric acid levels in rat blood which was measured quantitatively using uric acid enzyme kit was purchased from Coral Clinical Systems, U.S. Nagar, Uttarakhand, India.(16) For measuring the concentration of uric acid, 3 test tubes were taken and label as bank, standard, and sample. Then, 1.0 ml of working reagent (uricase) was added in each test tube and pre-warm all test tubes at 37 °C for at least 5 min. After pre-warming 25 µl sample and standard were added in sample and standard test tube respectively. All test tubes were incubated at 37 °C for minimum

of 10 min. After 10 min, absorbance of all prepared samples was measured at 520 nm using UV spectrophotometer against blank. A concentration of uric acid was measured using following equation:

$$\textbf{\textit{Uric Acid Concentration}} = \frac{Abs\ (sample)}{Abs\ (Standard)}\ X\ Concentration\ standard(mg/dl)$$

Equation- 9.2

9.5.2. X-ray

X-ray of rat's was taken at Angela Lobo clinic, Vadodara after harvesting it form rat after euthanizing humanly to study change in bone shape after completion of pharmacodynamic study. X-ray of all animal form all group were taken to study efficacy of given treatment.

9.6. Results and Discussion

9.6.1. Pharmacokinetic of Tacrolimus

LC-MS as described in chapter 3 section 3.4.2 was employed for the determination of concentrations of TAC in blood plasma of rats and the data are presented in Table 9.7. The data are also represented graphically in Fig. 9.1. Graph was plotted wherein Y-axis represented "TAC concentration in plasma" and X-axis represented "time".

Table 9.7: Plasma drug concentration of TAC after applying various formulation of TAC

Time (h)	Plasma drug concentration (ng/ml)				
	Marketed oral Tab	Transdermal film	Cubosomal gel	MN patch	Cubosomal loaded MN patch
0	0 ± 0.0	0 ± 0.0	0 ± 0.00	0 ± 0.00	0 ± 0.0
1	2.00 ± 0.50	1.21 ± 0.10	1.13 ± 0.10	1.44 ± 0.20	1.49 ± 0.26
2	2.43 ± 0.70	1.60 ± 0.30	1.58 ± 0.37	2.05 ± 0.30	2.07 ± 0.30

3	2.06 ± 0.30	2.00 ± 0.20	2.08 ± 0.45	1.82 ± 0.31	2.51 ± 0.30
4	1.81 ± 0.20	1.80 ± 0.30	2.39 ± 0.43	1.63 ± 0.29	2.95 ± 0.52
5	1.66 ± 0.40	1.55 ± 0.20	2.88 ± 0.47	1.52 ± 0.25	3.33 ± 0.55
6	1.41 ± 0.20	1.27 ± 0.20	2.53 ± 0.20	1.41 ± 0.21	3.80 ± 0.52
8	1.14 ± 0.20	1.01 ± 0.20	2.24 ± 0.30	1.14 ± 0.20	2.83 ± 0.37
12	0.83 ± 0.10	0.74 ± 0.20	1.74 ± 0.30	0.95 ± 0.19	2.35 ± 0.28
24	0.60 ± 0.09	0.57 ± 0.14	1.14 ± 0.30	0.67 ± 0.19	1.79 ± 0.41

n=3

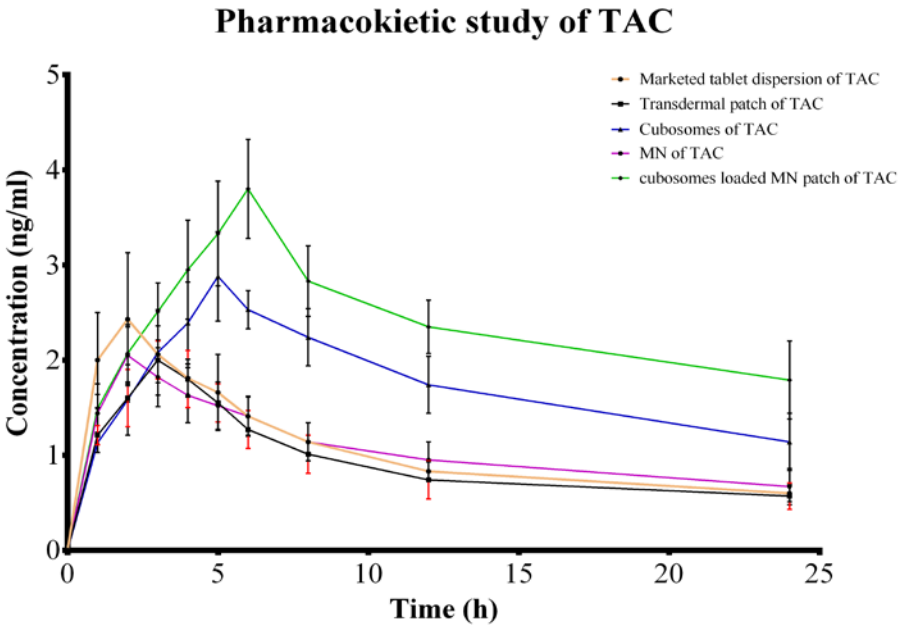

Figure 9.1: Plasma TAC concentration vs Time profile of various dosage forms in Sprague Dawley rats

Thermo Scientific™ Kinetica Software was utilized for the calculation of various PK parameters from the collected data and is summarized in Table 9.8.

Table 9.8: Pharmacokinetic parameters (TAC) computed using Kinetica Software

Parameters	Marketed oral Tablet	Transdermal film	Cubosomal gel	MN patch	Cubosomal loaded MN patch
C_{max} (ng/ml)	2.43	2.00	2.88	2.05	3.80
T_{max}	2.00	3.00	5.00	2.00	6.00
AUC_{0-t} (ng*h/ml)	34.72	30.61	66.78	46.10	120.81
$T_{1/2}$ (h)	12.03	11.86	16.03	21.48	25.43
MRT (h)	17.76	18.23	24.96	30.19	37.92
F_{rel}	1	0.88	1.92	1.33	3.48

Pharmacokinetic parameters of the formulation presented in table 9.8 were evaluated. When the marketed formulation of TAC i.e. suspension of tablet was administered through the oral route, higher concentration of TAC was recorded i.e. a C_{max} of 2.43 ng/mL and t_{max} of 2 hours but this was for a short duration of time ($t_{1/2}$ of 12.03 hours and MRT of 17.76 hours) over other formulations. It can be concluded from the above observation, that with such formulation, frequent dosing is required. Opposing to the trend seen in marketed suspension of tablet, transdermal formulation showed significantly better controlled plasma levels with sustained duration having a $t_{1/2}$ ranging from 11-26 hours and MRT ranging from 18-38 hours. Among all the formulation showed in table 9.8, transdermal film exhibited lowest C_{max} and AUC indicating poor penetrability of drug through intact skin. The poor penetrability of the transdermal film can be further supported by the fact that there was a significant improvement in C_{max} and AUC in cubosomes loaded in fast dissolving MN patch of TAC. However, when cubosomal gel of TAC and cubosomes loaded MN patch of TAC were compared, cubosomes loaded MN patch of TAC showed significant improvement in C_{max} and AUC than cubosomal gel of TAC. So, it can be concluded that cubosomes loaded MN patch of TAC has improved permeation. This is due to the fact this microneedles breach stratum corneum and drug reaches the systemic circulation. There was a significant improvement in C_{max} observed in the optimized transdermal formulations over suspension which was

administered orally. Also, there is a chance of dose reduction in developed formulations given to >3-fold rise in relative bioavailability in developed formulations. A synergistic effect is observed with cubosomes and MN patch, which shows highest permeation through skin. This indicates the possibility of reduction in dose and consequently the side effects.

9.6.2. Pharmacokinetic of Febuxostat

HPLC as described in chapter section 3.5.1 was employed for the determination of concentrations of FBX in blood plasma of rats and the data are presented in Table 9.9. The data are also represented graphically in Fig. 9.2. Graph was plotted wherein Y-axis represented “FBX concentration in plasma” and X-axis represented “time”.

Table 9.9: Plasma drug concentration of FBX after applying various formulation of FBX

Time (h)	Plasma drug concentration (ng/ml)				
	Marketed oral Tab	**Transdermal film**	**Cubosomal gel**	**MN patch**	**Cubosomal loaded MN patch**
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
1	140.67 ± 26.5	30.37 ± 9.2	57.27 ± 7.2	62.04 ± 11.4	70.76 ± 9.2
2	207.53 ± 33.9	48.62 ± 10.7	80.97 ± 13.9	102.38 ± 20.2	97.64 ± 18.1
3	187.05 ± 26.2	76.27 ± 11.1	122.84 ± 16.7	114.10 ± 14.2	139.28 ± 24.9
4	153.01 ± 19.6	97.58 ± 14.5	159.33 ± 26.3	107.55 ± 20.6	181.88 ± 34.4
5	132.14 ± 15.7	65.90 ± 10.3	191.64 ± 27.2	97.66 ± 11.2	212.82 ± 34.7
6	121.99 ± 13.0	48.31 ± 11.1	218.44 ± 42.3	89.53 ± 15.8	271.03 ± 50.3
8	101.69 ± 8.4	37.89 ± 6.6	191.11 ± 28.7	75.41 ± 7.91	240.06 ± 34.4
12	69.94 ± 3.7	23.94 ± 5.5	122.57 ± 22.5	58.04 ± 9.91	185.47 ± 28.8
24	42.55 ± 4.5	7.17 ± 2.5	64.67 ± 18.2	32.57 ± 4.89	121.86 ± 20.0

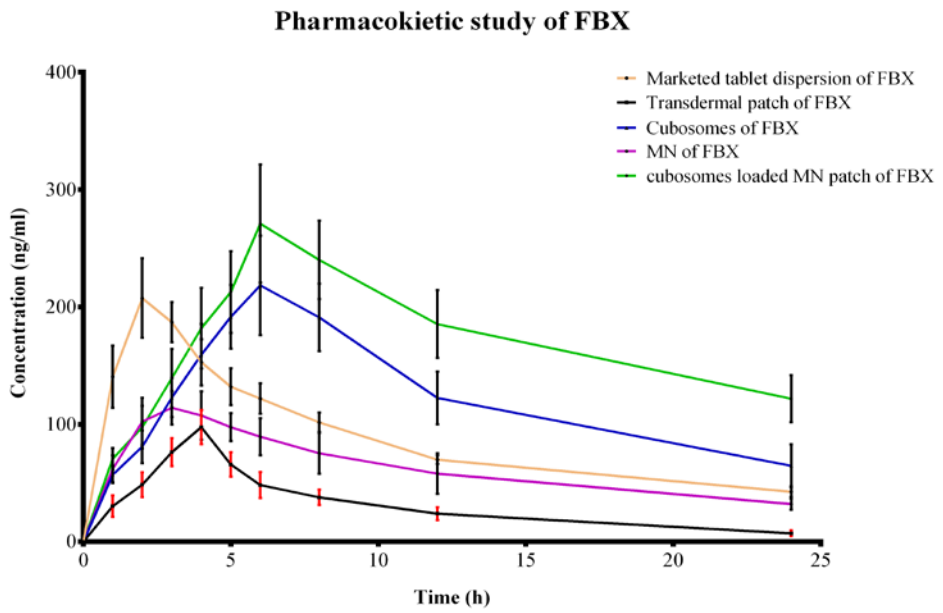

Figure 9.2: Plasma FBX concentration vs Time profile of various dosage forms in Sprague Dawley rats

Thermo Scientific™ Kinetica Software was utilized for the calculation of various PK parameters from the collected data and is summarized in Table 9.10.

Table 9.10: Pharmacokinetic parameters (FBX) computed using Kinetica Software

Parameters	Marketed oral Tablet	Transdermal film	Cubosomal gel	MN patch	Cubosomal loaded MN patch
C_{max} (ng/ml)	207.53	97.58	218.44	112.48	271.03
T_{max}	2.00	4.00	6.00	5.00	6.00
AUC_{0-t} (ng*h/ml)	2784.06	798.84	3825.48	1957.89	7328.70
$T_{1/2}$ (h)	11.72	6.32	11.28	11.15	18.57
MRT (h)	16.91	9.79	18.43	17.72	29.05
F_{rel}	1	0.29	1.37	0.70	2.63

Pharmacokinetic parameters of the formulation presented in table 9.10 were evaluated. When the marketed formulation of FBX i.e. suspension of tablet was administered through the oral route, higher concentration of FBX was recorded i.e. a C_{max} of 207.53 ng/mL and t_{max} of 2 hours but this was for slightly longer hours ($t_{1/2}$ of 11.72 hours and MRT of 16.91 hours) comparable to other formulations. It can be concluded from the above observation, that with such formulation, frequent dosing is required. Opposing to the trend seen in marketed suspension of tablet, transdermal formulation showed significantly better controlled plasma levels with sustained duration having a $t_{1/2}$ ranging from 11-19 hours and MRT ranging from 18-29 hours. Among all the formulation showed in table 9.10, transdermal film exhibited lowest C_{max} and AUC indicating poor penetrability of drug through intact skin. The poor penetrability of the drug from the transdermal film can be further supported by the fact that there was a significant improvement reported in C_{max} and AUC in cubosomes loaded in fast dissolving MN patch of FBX. However, when cubosomal gel of FBX and cubosomes loaded MN patch of TAC were compared, cubosomes loaded MN patch of FBX showed significant improvement in C_{max} and AUC than cubosomal gel of FBX. So, it can be concluded that cubosomes loaded MN patch of FBX has improved permeation. This is due to the fact this microneedles breach stratum corneum and drug reaches the systemic circulation. There was a significant improvement in C_{max} observed in the optimized transdermal formulations over suspension which was administered orally. Also, there is a chance of dose reduction in developed formulations given to nearly 2.5-fold rise in relative in developed formulations. A synergistic effect is observed with cubosomes and MN patch, which shows highest permeation through skin. This indicates the possibility of reduction in dose and consequently the side effects.

9.6.3. Pharmacodynamic of Tacrolimus

9.6.3.1. Paw volume determination

Measured and reported paw volume of rats is given in table 9.11 and depicted in fig 9.3. Paw volume of group 2 animals were found to be highest in all groups which is disease control model. This proves that RA can be induced in rat using CFA.(11) From the fig 9.3 and data given in table 9.11 it can be concluded

that there was a decrease in the paw volume of rats of group 3 & 4 which had continued treatment as compared to group 2, which wasn't given any treatment. This proved the efficacy of the prepared formulation for the treatment of RA. However, no major difference was observed in the paw volume of rats of groups 3 & 4, which suggest that efficacy of the prepared formulation is comparable to that of the marketed product.

Table 9.11: Paw volume determination in pharmacodynamic model of RA

Group No.	Paw volume (ml)	SD
1	1.07	0.23
2	3.33	0.31
3	1.77	0.32
4	1.73	0.21

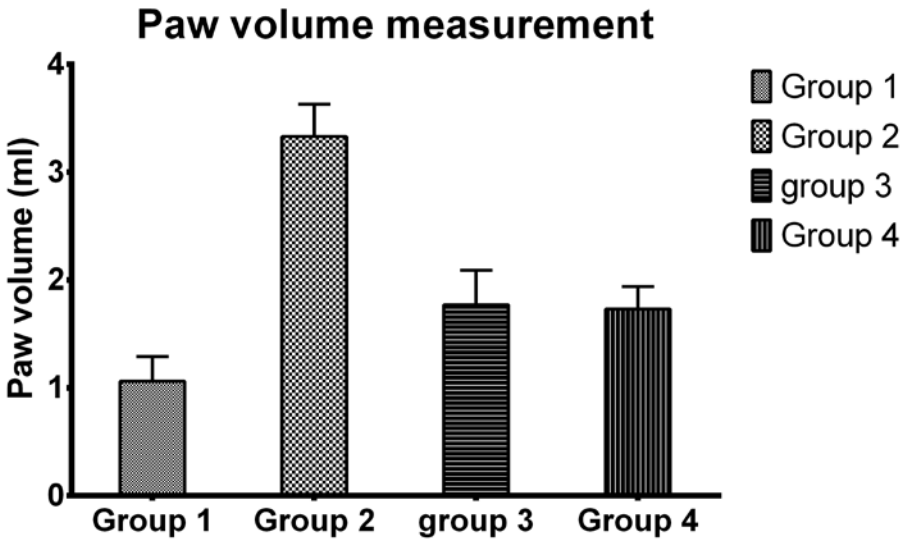

Figure 9.3: Paw volume determination for RA. Group 1-Normal control, Group-2 Model control, Group-3 Standard control, Group 4- Test Control

9.6.3.2. Body weight determination

Body weight of rats and the difference in their final and intial weight was calculated and listed in table 9.12 and depicted in fig 9.4. From the data obtained, it can be observed that the weight of rats in the disease control model (Group 2)

decreased drastically as compared to the normal model (Group 1), which suggests successful induction of disease in rats. Group 3 & 4 were treated with marketed and proposed formulation respectively, and show less weight reduction as compared to Group 2, which proved that prepared formulation is as effective as marketed formulation.

Table 9.12: Determination of body weight change during therapy in pharmacodynamic model of RA

Group No.	Change in body weight (gm)	SD
1	7.66	2.08
2	-45	10.44
3	-15.33	1.53
4	-15	3.61

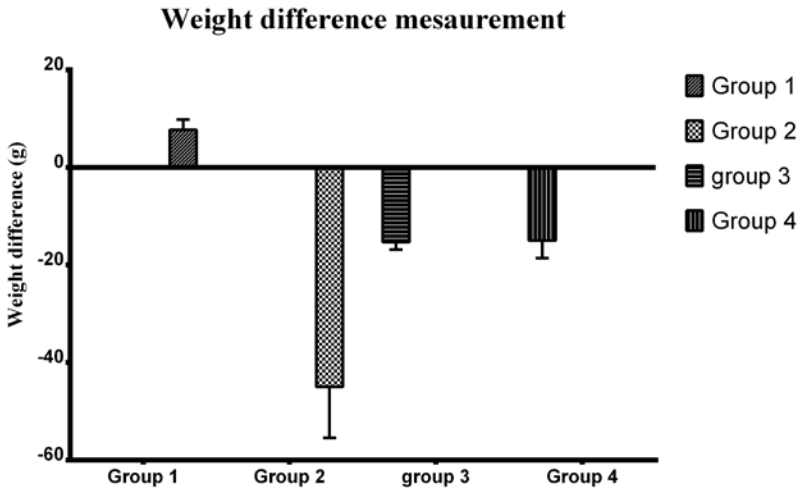

Figure 9.4: Measurements of weight gain/loss during the pharmacodynamic study of TAC. Group 1-Normal control, Group-2 Model control, Group-3 Standard control, Group 4- Test Control

9.6.3.3. Measurement of Rh factor

When RA was induced in rat model, there was an increase in Rh factor in rat blood which was found to be highest in Group 2 (disease model) and lowest in Group 1 (normal group) as shown in table 9.13 and depicted in figure 9.5. RF

factor in Group 3 & 4 are nearly same and less than Group 2 which suggests the effectively of the developed formulation.

Table 9.13: Absorbance for preparing calibration plot of calibrator RF

Sr. No.	Calibrator RF (µl)	Absorbance
1	25	0.034 ± 0.002
2	50	0.06 ± 0.001
3	100	0.126 ± 0.006
4	200	0.229 ± 0.008
5	400	0.429 ± 0.01

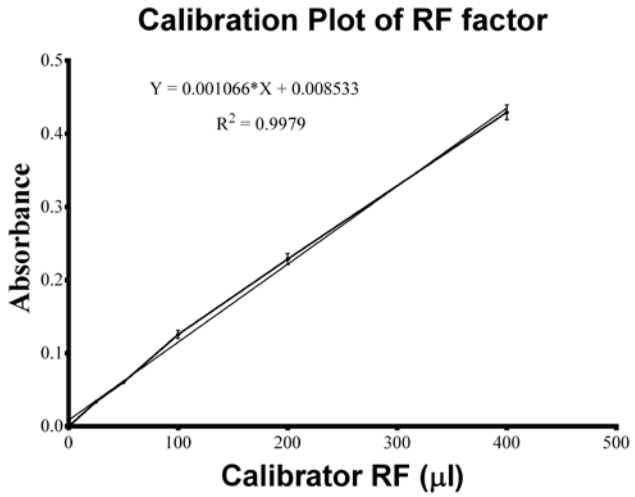

Figure 9.5: Calibration plot for RF factor to find out RF factor in rat serum

Table 9.14: Determination of Rheumatoid factor in rat serum

Group No.	Rheumatoid factor in rat serum	SD
1	14.23	2.36
2	162.13	3.01
3	40.18	4.23
4	42.06	3.79

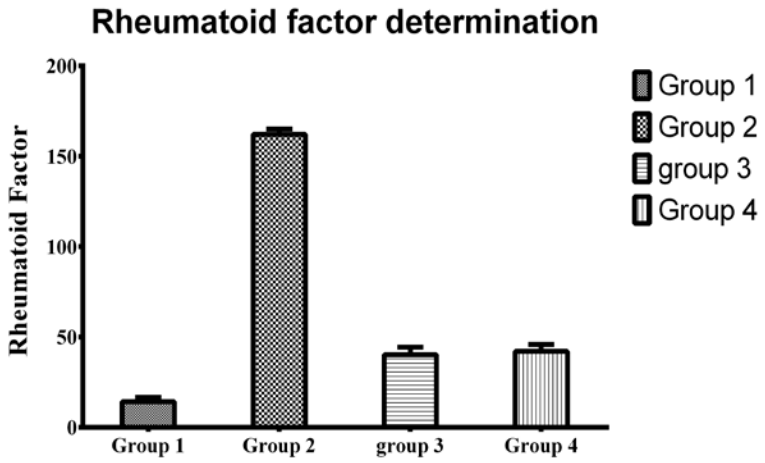

Figure 9.6: Measurement of Rheumatoid factor in rat serum. Group 1-Normal control, Group-2 Model control, Group-3 Standard control, Group 4- Test Control

9.6.3.4. X-ray

Bone X-rays of rats from all groups are depicted in fig 9.7. Comparison of X-rays of group 1 and group 2 clearly shows that Group 2 has a more abnormal bone X-ray as compared to group 1. Moreover, bone X-rays of Group 3 & 4 were also found normal and comparable to the bone X-ray of Group-1. This proves the efficacy of the prepared cubosomal loaded MN patch and is comparable to the marketed formulation of TAC.

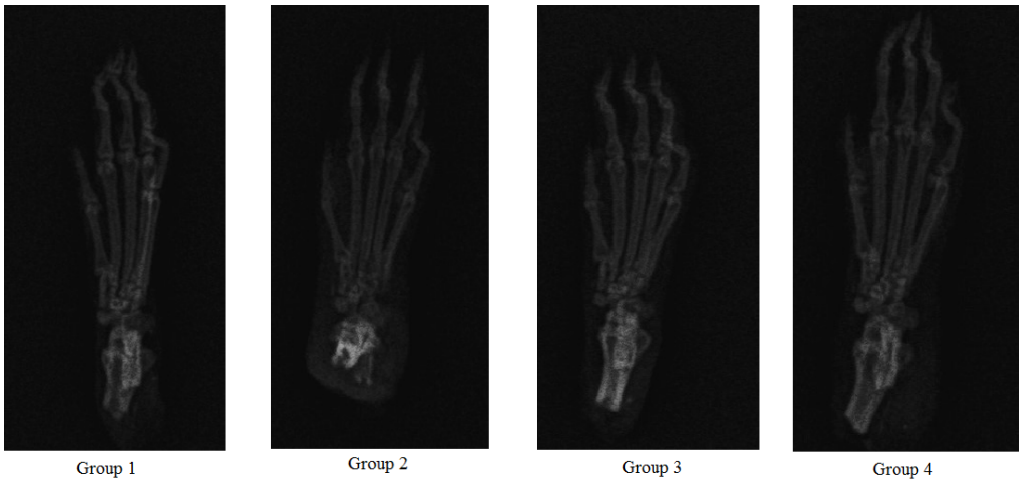

Figure 9.7: X-ray of rat's paw to study the effectiveness of developed formulation of TAC

9.6.4. Pharmacodynamic of Febuxostat

9.6.4.1. Measurement of Uric Acid

While inducing gout in rat model, there was an increase in uric acid levels in rat blood which was further converted into monosodium urate crystals and these crystals deposited in various joints of bones and caused Gout. It was found highest in Group 2 (disease model) and lowest in Group 1 (normal group). This high level of uric acid in rats of Group 2 suggests the induction of hyperuricemia in rats as listed in table 9.17 and depicted in fig 9.10. Uric acid levels in Group 3 & 4 were nearly same and less than Group 2 which suggests the efficacy of the developed formulation.

Table 9.15: Determination of Uric acid level in rat serum

Group No.	Uric acid level in serum (mg/dl)	SD
1	1.25	0.10
2	4.90	0.23
3	2.18	0.12
4	2.05	0.12

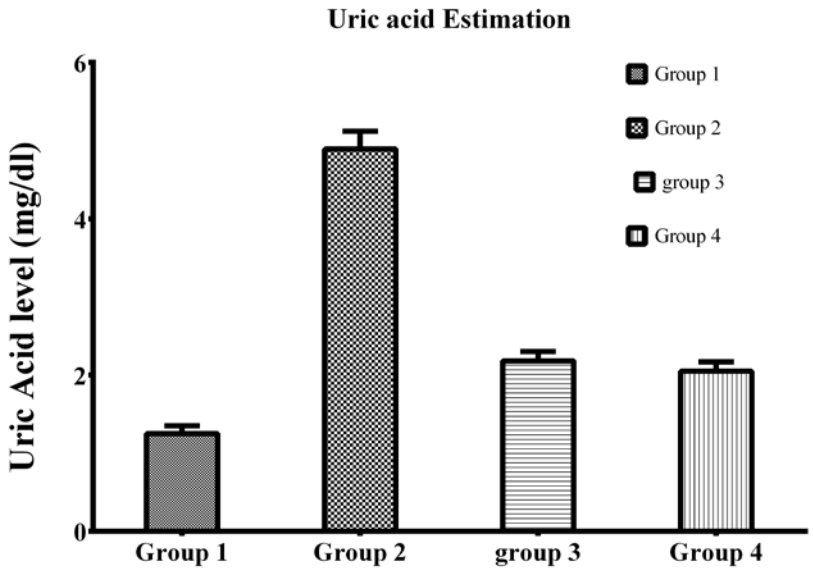

Figure 9.8: Measurement of serum uric acid level in rat serum. Group 1-Normal control, Group-2 Model control, Group-3 Standard control, Group 4- Test Control

9.6.4.2. X-ray

Bone X-rays of rats from all groups are depicted in fig 9.11. All X-rays obtained during study, were same by which we can conclude that there is no significant difference between the X-rays of all groups.

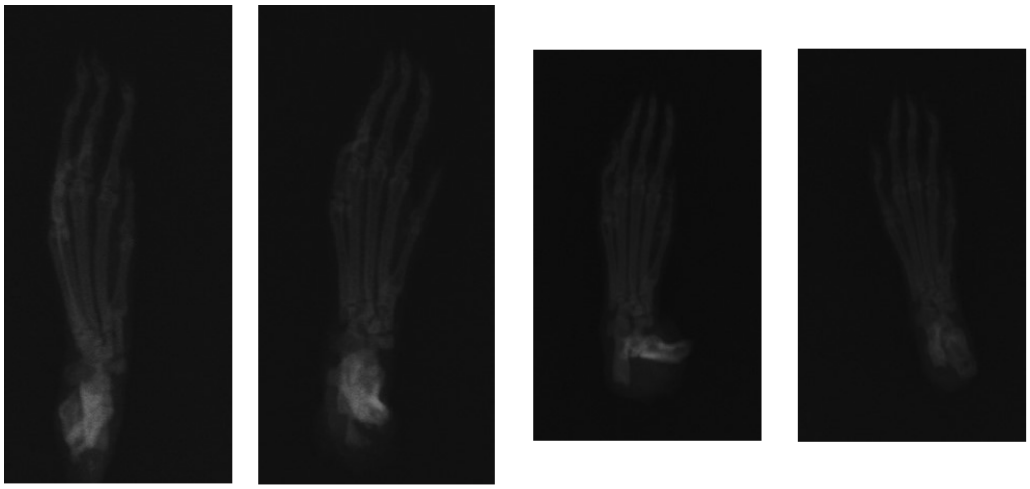

Figure 9.9: X-ray of rat's paw to study the effectiveness of developed formulation of FBX

During induction of gout in rats, no significant difference was found in paw volume or weight of rats. Moreover, bone X-rays of rats also did not confirm the formation of tophi. Apart from this, according to the literature, induction of gout tophi requires years of induction treatment, and chances of production of gout tophi are also very less. However, increased level of uric acid suggests the induction of hyperuricemia and this increased level of uric acid in blood is responsible for the induction of gout according to the literature.(17) This uric acid level was controlled using developed and marketed formulation and proved the efficacy of therapy.

9.7. References

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