Assessment of Pharmacokinetic parameters and antihypertensive activity of prepared sildenafil transdermal patches

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Abstract

The aim of present study was to prepare and evaluate sildenafil transdermal patches for antihypertensive activity, which can be beneficial and convenient for the children who have been connected with Pediatric Pulmonary arterial hypertension and In this project, we investigated the antihypertensive effect of the formulated patches in deoxycorticosterone acetate (DOCA)–salt rat model. Antihypertensive activity was evaluated in sildenafil patches for 4 weeks in DOCA treated rats. Blood pressure by non-invasive (indirect) method and invasive method was measured. Administration of formulated patches for 4 weeks in DOCA treated rats significantly ($p<0.05$) reduced the mean arterial blood pressure and vascular reactivity changes to various catecholamines. Results of the present work suggest that formulated patches has an antihypertensive action in unilateral nephrectomized DOCA-salt hypertensive rats and could be possible starting point for treatment of hypertension with increased patient adherence.

Keywords: Transdermal; Skin permeation enhancer; *In vitro; In Vivo, Sildenafil;* Transdermal delivery, Pediatric pulmonary arterial hypertension (PAH).

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Introduction

Pulmonary hypertension (PH) is a disease characterized by elevated pulmonary artery pressure, which can result in right ventricular failure. In children, PH is most commonly associated with underlying cardiac or lung disease (eg, bronchopulmonary dysplasia [BPD]). Pediatric pulmonary arterial hypertension (PAH) shares common features of adult disease, Pediatric Pulmonary arterial hypertension is a rare blood vessel disorder of the lung in which the pressure in the pulmonary artery (the blood vessel that leads from the heart to the lungs) rises above normal levels [1, 2]. An increase of the number of smooth muscle cells in the walls of small lung arteries (a phenomenon called proliferation) that are remodeling the vessels, may lead to obstructions in the microcirculation, which will then lead to an increase in the blood pressure[3]. Chronic thromboembolic pulmonary hypertension is a complication representing less than 1% of all cases of acute pulmonary embolism (the sudden blocking of a lung artery by a clot or foreign material which has been brought to its site by the blood current), which directly leads to pulmonary hypertension[4, 5]. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension are chronically debilitating and life-threatening [6]. Sildenafil protects cyclic guanosine monophosphate (cGMP) from degradation by cGMP-specific phosphodiesterase type 5 (PDE5) in the corpus cavernosum [7]. Nitric oxide (NO) in the corpus cavernosum of the penis binds to guanyl ATBCyclase receptors, which results in increased levels of cGMP, leading to smooth muscle relaxation (vasodilation) of the intimal cushions of the helicine arteries[8, 9]. This smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis, causing an erection. Robert F. Furchgott, Ferid Murad, and Louis Ignarro won the Nobel Prize in Physiology or Medicine in 1998 for their independent study of the metabolic pathway of nitric oxide in smooth muscle vasodilation. The molecular mechanism of smooth muscle relaxation involves the enzyme CGMP-dependent protein kinase, also known as PKG [10, 11]. This kinase is activated by cGMP and it phosphorylates multiple targets in the smooth muscle cells, namely myosin light chain phosphatase, RhoA, IP3 receptor, phospholipase C, and others. Overall, this results in a decrease in intracellular calcium and desensitizing proteins to the effects of calcium, engendering smooth muscle relaxation [12]. The present study aimed to formulation of sidinafile citrate patches for pharmacological activity.

1. Material and Methods

Animals

Wistar rats weighing 180–220 grams were used for this experimental examination. Rats comprise procured through the animal house facility regarding the institute. Animals had been kept in nicely-ventilated polypropylene cages under a surrounding temperature of 25 ± 2 ℃, 12-h light/ dark cycle inside the animal house that is departmental.

Chemicals

Urethane, and DOCA were purchased from Sigma-Aldrich, Mumbai. All drug solutions were freshly prepared in saline before each experiment.

Formulation Design and manufacturing process:

Table 1: Following formulation composition were subjected for the in-vivo study in order to explore the pharmacokinetic and antihypertensive activities.

Sr. No.	Ingredient	Function	FA1	FT1
1	Sildenafil Citrate	Active	75 mg	75 mg
2	Acrylic polymer	Adhesive agent	400 mg	300 mg
3	TEC	Plasticizer	10%	10%
4	Isopropyl myristate	Permeation enhancer	--	20%
5	TEC (mg)		40	30
6	Isopropyl myristate (mg)		--	60
Total Quantity (mg)			**515**	**465**

Preparation of transdermal patches and evaluation (Single-layer drug in adhesive method):

Step 1: Selected polymer was weighed and then completely dispersed into 8 ml of water, then this polymeric solution was kept aside for two hrs.

Step 2: As a part of drug solution preparation, drug was dissolved into 2 ml ethanol.

Step 3: Drug solution of Step 2 was added into above polymeric solution of Step 1

Step 4: Mixture of those drug and polymeric solution was stirred until clear dispersion form.

Step 5: Plasticizer was added into the step 4 to get the proper elasticity in the transdermal patch.

Step 6: This clear dispersion finally poured in the petri plate, which was previously lubricated with tween 80.

Step 7: This Petri plate was dried at room temperature in dark condition for overnight period and in order to get uniform evaporation of the solvent, an inverted funnel was kept on the petri plate.

Step 8: The dried films were removed from the petri-plate and the patches were cut into 4 cm^2 area.

Step 9: Biaxial oriented polyethylene film was used as a backing membrane and glossy paper was used as a release liner.

Step 10: At last, the prepared patches were stored in desiccators for further evaluation purpose.

Table 2 : *In-vitro* drug release and *In-vitro* permeation study profile of sildenafil citrate from transdermal patches

TIME (h)	Cumulative % drug release	
	FA1	FT1
1	10± 1.73	9± 0.75
4	15± 1.72	15± 0.55
8	36± 1.51	28± 0.45
12	45± 1.21	40± 0.36
18	75± 1.52	69± 0.65
24	85± 1.50	78± 0.70
48	98± 1.21	90± 0.32

TIME (h)	Cumulative amount of drug permeated (µg/cm2/hr)	
	FA1	FT1
1	4.42 ± 0.42	8.59 ± 0.89
4	75.53 ± 6.98	115.21 ± 2.23
8	157.06 ± 7.45	226.39 ± 4.15
12	285.92 ± 13.22	343.80 ± 3.67
18	338.55 ± 18.42	489.75 ± 7.12
24	427.78 ± 13.76	560.29 ± 3.76
48	850.68 ± 20.56	1012.29 ± 6.11

2. Experimental design for Assessment of Bio distribution of drugs in various organs

Before the dosing with test drug, the rats were fasted for 12 hours. During fasting, the drinking water was abstained.

The animals will be randomly divided into four groups (n-6)

Group 1: Control group (placebo-water),

Group 2: Test group (Test formulation A),

Group 3: Test group (Test formulation B)

Group 4: Standard group (Pure drug).

Patches of test formulation will be applied on dorsal surface shaved skin of respective group of animals. Standard drug suspension was prepared by using 0.5% CMC and administrated orally by using oral gavage to respected group of animals. Animals belonging to aforesaid groups (n = 6) will be sacrificed via cervical dislocation at their respective time point post dosing. Major organs will be collected, washed, weighed. The samples will be prepared by mobile phase/phosphate buffer to each and homogenized. The homogenized samples were sonicated for 5 min. After vortexing for 30 s a protein precipitating agent was added and vortexed for 1 min. The mixture was centrifuged at 7000 RPM for 15 min. The supernatant will be filtered through syringe driven membrane filter unit and of the filtrate will be injected onto the HPLC system. Parameters was evaluated "Comparison of difference in drug concentration in major organs and in plasma at different time points"[13, 14].

3. Antihypertensive activity of formulation

Animal

Female albino rats (Wistar strain) weighing between 150 and 200 g were obtained from Central animal house facility. Animals were housed into groups of five under standard laboratory conditions of temperature 25°C±1°C with free access to food and water. The experiments were performed during the light portion (9–14 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and approved by the Institutional Animal Ethical Committee.

Induction of DOCA- Salt induced hypertension

Induction of DOCA- Salt induced hypertension involves anesthesia by ketamin (75 mg/kg; i.p) and xylazine (7.5 mg/kg; i.p) and uninephrectomy via left flank incision. A week after unilateral nephrectomy, DOCA (25mg/kg, once a week; s.c; for 4 weeks) dispersed in cotton seed oil was

injected to uninephrectomised rats. 1% saline and 0.2% KCl ad libitum were given throughout the experiment instead of drinking water [15].

4. Experimental protocol for formulation

Group I: Sham Control, Unilateral nephrectomized animals receive daily injection of 0.1ml of sterilized cotton seed oil subcutaneously for 4weeks and 0.2% KCl adlibitumas drinking water.

Group II: Unilateral nephrectomized animals receive formulation A (12 mg/kg) for 4 weeks and 1% saline and 0.2% KCl adlibitumas drinking water.

Group III: Unilateral nephrectomized animals receive formulation B (12 mg/kg) for 4 weeks and 1% saline and 0.2% KCl adlibitumas drinking water.

Group IV: Unilateral nephrectomized animals receive DOCA injection (25 mg/kg/week, s.c.) for 4weeks, dissolved in sterilized cotton seed oil subcutaneously and 1% saline and 0.2% KCl adlibitumas drinking water.

Group V: Unilateral nephrectomized animals receive DOCA injection (25 mg/kg/week, s.c.), formulation A (12 mg/kg) for 4 weeks and 1% saline and 0.2% KCl adlibitumas drinking water.

Group VI: Unilateral nephrectomized animals receive DOCA injection (25mg/kg/week,s.c.),formulation B (12 mg/kg) for 4 weeks and 1% saline and 0.2% KCl ad libitumas drinking water.

Measurement of blood pressure

Measurement of blood pressure by noninvasive (indirect) method

The rats were trained for at least one week until the BP is steadily recorded with minimal stress and restrain. The first cardiovascular parameters were discarded and mean of five or six subsequent measurements were recorded. Systolic blood pressure is measured weekly for four weeks by indirect non-invasive tail-cuff method using Power Lab [16].

Measurement of blood pressure by invasive (direct) method

After completion of treatment schedule rats from each group were anesthetized with urethane (120mg/100gm). Femoral vein is cannulated with fine polyethylene catheter for administration of the drug. Tracheostomy is performed and blood pressure is recorded from left common carotid artery using pressure transducer by direct method on Chart data system. Heparinized saline (100 IU/ml) is filled in the transducer and in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, heart rate, basal blood pressure was recorded[17].

5. Result and Discussions

Pharmacokinetic study of test formulation A (FT1) and B (FA1) (Transdermal patches)

In this study, we have determined mean plasma concentration of formulation A (FT1) and B (FA1) in compared with standard drug. We found that formulation of transdermal patches A (FT1) and B (FA1) have better plasma concentration as compared to the standard drug shown in figure 1 and table 3.

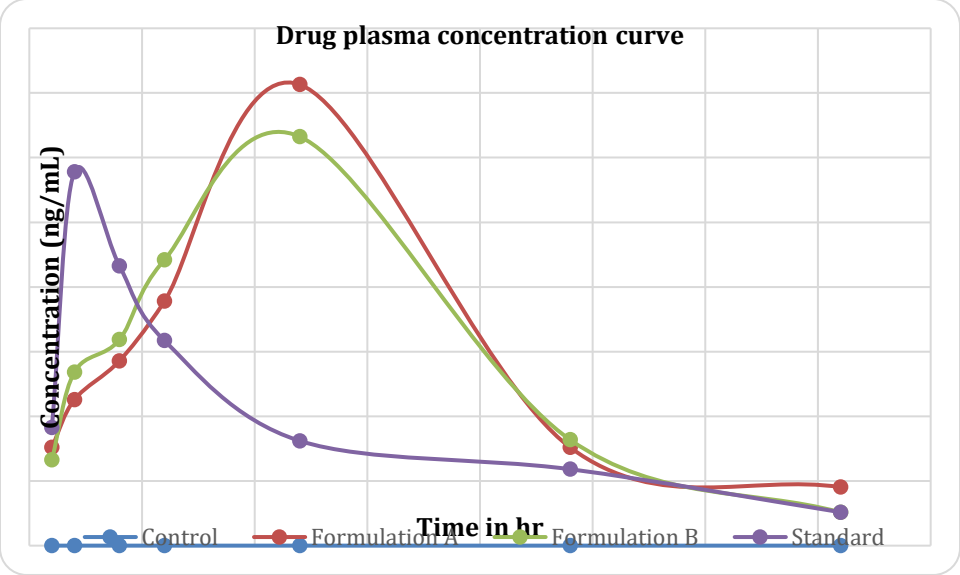

Figure 1-Mean Plasma concentration

Table 3: Plasma concentration of Sildenafil Citrate

Standard					**Formulation A (FT1)**					**Formulation B (FA1)**				
Time	Conc	C1-C2	Correction factor	AUC	Time	Conc.	C1-C2	Correction factor	AUC	Time	conc	C1-C2	Correction factor	AUC
0	0	--	--	--	0	0	--	--	--	0	0	--	--	--
2	3.650	2	1.8250	1.519633	2	3.039266	2	1.5196329	1.519633	2	2.653	2	1.3265	1.519633
4	11.560	2	5.7800	7.299633	4	4.513975	2	2.2569875	3.776620	4	5.365	2	2.6825	4.202133
8	8.654	4	2.1635	9.463133	8	5.715246	4	1.4288116	5.205432	8	6.370	4	1.5925	5.794633
12	6.340	4	1.5850	11.048130	12	7.558543	4	1.8896356	7.095068	12	8.840	4	2.2100	8.004633
24	3.240	12	19.4400	30.488130	24	14.259023	12	66.765530	73.860600	24	12.648	12	75.8880	83.89263
48	2.364	24	28.368	58.856133	48	3.038585	24	36.463016	110.3236	48	3.275	24	39.3	123.1926
72	1.034	48	24.816	83.672133	72	1.80892	48	43.414069	153.7337	72	1.03	48	24.72	147.9126

Cmax, AUC and Tmax

In the Cmax, formulation A (FT1) it was found 14.26 ng/mL and formulation B (FA1) 12.65 ng/mL. Cmax of standard was 3.24 ng/mL. From this study we found that formulation A (FT1) has better Cmax than the formulation B (FA1) and standard shown in below table. In the AUC, formulation A (FT1) it was found 153.73 ng.hr/mL and formulation B (FA1) 147.91 ng.hr/mL. AUC of standard was 83.67 ng.hr/mL. From this study we found that formulation A (FT1) has better AUC than the formulation B (FA1) shown in below table. Tmax of formulation A (FT1) was found 24 hr and in case of formulation B (FA1) it was 24 hr, meanwhile in standard group it was 4 hr. Data of Tmax given below in table.

Table 4: Cmax, AUC and Tmax of formulation A (FT1) and B (FA1)

Sample	Cmax (ng/mL)	AUC (ng.hr/mL)	Tmax (hr)
Formulation A (FT1)	14.26	153.73	24
Formulation B (FA1)	12.65	147.91	24
Standard	3.24	83.67	4

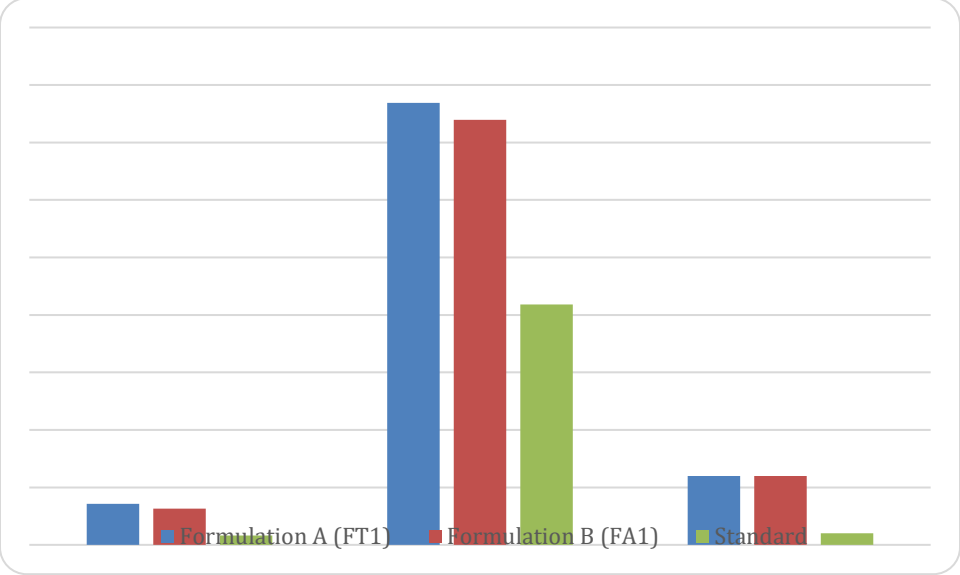

Figure 2: Cmax, AUC & Tmax of formulation A (FT1) and B (FA1)

Biodistribution in various organs of test formulation A (FT1) and B (FA1) (Transdermal patches)

We have determined concentration of drug in various organs like lung, kidney, liver, fats, brain, pancreas, heart, small intestine, and muscle at different time interval and the result

shown in the table 5.

Table 5: concentration of drug in various organs muscle at different time interval

Name of Organ	**Concentration of drug found in organs of control group (µg)**							
	5 hr	24 hr	48 hr	72 hr				
Lung	--	--	--	--				
Liver	--	--	--	--				
Heart	--	--	--	--				
Spleen	--	--	--	--				
Stomach	--	--	--	--				
Brain	--	--	--	--				
Small intestine	--	--	--	--				
Muscle	--	--	--	--				
Fat	--	--	--	--				
Kidney with adrenal gland	--	--	--	--				
Large intestine	--	--	--	--				
Name of Organ	**Concentration of drug found in organs of Test formulation A (FT1) (µg)**				**Concentration of drug found in organs of Test formulation B (FA1) (µg)**			
	5 hr	24 hr	48 hr	72 hr	5 hr	24 hr	48 hr	72 hr
Lung	--	10±1.2	--	--	--	--	--	--
Liver	26±2.1	--	--	28±1.8	--	--	13±1.8	22±2.9
Heart	--	--	--	--	--	--	--	16±2.8
Spleen	--	--	--	--	--	--	--	--
Stomach	3±0.3	7±0.4	16±1.5	16±1.5	3±0.9	5±0.4	13±1.6	15±3.2
Brain	--	--	--	--	--	--	--	--
Small intestine	--	--	--	--	--	--	--	--
Muscle	--	--	--	--	--	--	--	--
Fat	72±4.8	93±5.4	103±8.5	123±5.2	26±1.7	48±3.7	79±4.8	103±7.9
Kidney with	--	--	--	--	13±1.	31±2.	42±3.	56±5.

adrenal gland					2	1	5	4
Large intestine	--	--	--	13 ± 2.9	- -	- -	- -	- -

Conclusion: Based on the above study, it was concluded that the organ of the rat was subjected with the test formulation A (FT1) and B (FA1) were detected the drug in various organs like lung, liver, heart, stomach, fat and kidney. This data reveled that the drug was permeated through the skin and reached the various organs through systemic blood circulation.

Measurement of blood pressure by non-invasive (indirect) method after administration of formulation

Administration of Deoxycorticosterone acetate (DOCA) for 4 weeks in unilateral nephrectomized rats produced a significant elevation ($p<0.05$) in systolic blood pressure (SBP) as measured by tail cuff method on II, III and IV week when compared to sham control rats. Unilateral nephrectomized rats which received formulation A (FT1) and B (FA1) for 4 weeks along with DOCA significantly ($p<0.05$) reduced SBP on III and IV week as compared with SBP of unilateral nephrectomized DOCA-salt hypertensive rats, thus implying an antihypertensive effect. However, chronic administration of formulation A and B in unilateral nephrectomized rats for 4 weeks did not alter SBP as compared to sham control.

Table 6: Effect of formulation mg/kg/day, p.o., for 4 weeks) on SBP in DOCA- salt hypertensive rats

Treatment groups (mg/kg)	**Mean SBP (mm Hg)**				
	0 week	**I week**	**II week**	**III week**	**IV week**
Sham control	105.7 ± 2.8	107.7 ± 2.3	108.5 ± 1.8	111.7 ± 2.2	113.4 ± 2.9
Formulation A (FT1)	104.2 ± 3.1	106.4 ± 3.4	108.6 ± 1.7	107.6 ±2.5	113.5 ± 2.6
Formulation B (FA1)	106.5 ± 2.2	107.4 ± 2.1	107.6 ±1.2	108.6 ± 3.1	107.9 ± 2.1
DOCA (25)	105.7 ± 2.6	108.5 ± 2.6	111.5 ± 1.4	118.5 ± 3.5	119.8 ± 2.5
DOCA (25)+ Formulation A (FT1)	103.8 ± 3.6	105.6 ± 2.5	106.5 ± 1.3	107.8 ± 3.6	109.5 ± 3.0
DOCA (25)+ Formulation B (FA1)	106.4 ± 2.4	107.2 ± 3.7	108.4 ± 1.7	107.9 ± 3.2	108.9 ± 3.5

*All values were expressed as mean ± SEM, n=5. All data were subjected to one way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to sham control and # $p<0.05$ when compared to DOCA group.*

Measurement of blood pressure by invasive (direct) method

The heart rate, basal arterial blood pressure and pressor responses to NA, Adr, PE, 5- HT and Ang II were significantly ($p<0.05$) increased in unilateral nephrectomized DOCA-salt hypertensive rats as compared to sham control rats. The heart rate, basal arterial blood pressure and pressor responses to NA, Adr, PE, 5-HT and Ang II were significantly ($p<0.05$) reduced in case of unilateral nephrectomized DOCA-salt hypertensive rats that received formulation A (FT1) and B (FA1) for 4 weeks as compared to unilateral nephrectomized DOCA-salt hypertensive rats. The heart rate, basal arterial blood pressure and pressor responses to NA, Adr, PE, 5-HT and Ang II were not altered in case of Formulation A (FT1) and B (FA1) treated unilateral nephrectomized rats as compared to sham control rats.

Table 6: Effect of formulation on heart rate (BPM) and basal arterial blood pressure in DOCA-salt hypertensive rats

Treatment groups (mg/kg)	Basal arterial blood pressure (mm Hg)	Heart rate (BPM)
Sham control	97.63 ± 4.74	328 ± 4.67
Formulation A (FT1)	92.0± 2.04	330 ± 7.82
Formulation B (FA1)	98.5 ± 0.50	370 ± 8.42
DOCA (25)	138 ± 4.79*	397± 9.58*
DOCA (25)+ Formulation A (FT1)	96.5± 3.20#	318± 9.14#
DOCA (25)+ Formulation B (FA1)	97.3 ± 2.51#	323 ± 6.12#

*All values were expressed as mean ± SEM, n=5. All data were subjected to One Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to sham control and # $p<0.05$ when compared to DOCA group.*

6. Conclusion:

The Cmax of the formulation A (FT1) was 14.26 ng/mL and formulation B (FA1) was 12.65 ng/mL. Cmax of standard was 11.56 ng/mL. This study revealed that the formulation A (FT1) has better Cmax than the formulation B (FA1) and standard. The AUC of the formulation A (FT1) was 153.73 ng.hr/mL and formulation B (FA1) was 147.91 ng.hr/mL. AUC of standard was 83.67 ng.hr/mL. Based on this study it was concluded that the formulation A (FT1) has better AUC than

the formulation B (FA1). Tmax of formulation A (FT1) was found 24 hr and in case of formulation B (FA1) it was 24 hr, meanwhile in standard group it was 4 hr.

As a part of assessment of Bio distribution of drugs in various organs, patches of test formulation were applied on dorsal surface shaved skin of respective group of animals. Standard drug suspension was prepared by using 0.5% CMC and administrated orally by using oral gavage to respected group of animals. Animals belonging to aforesaid groups (n = 6) were sacrificed via cervical dislocation at their respective time point post dosing. Major organs were collected, washed, weighed. The samples were prepared by mobile phase/phosphate buffer to each and homogenized. The homogenized samples were sonicated for 5 min. After vortexing for 30 sec a protein precipitating agent was added and vortexed for 1 min. The mixture was centrifuged at 7000 RPM for 15 min. The supernatant was filtered through syringe driven membrane filter unit and of the filtrate was injected onto the HPLC system. Parameters was evaluated "Comparison of difference in drug concentration in major organs and in plasma at different time points".

Based on the bio distribution of drugs in various organs study, it was concluded that the organ of the rat was subjected with the test formulation A (FT1) and B (FA1) were detected the drug in various organs like lung, liver, heart, stomach, fat and kidney. This data reveled that the drug was permeated through the skin and reached the various organs through systemic blood circulation.

Measurement of blood pressure study were conducted in two methods i.e. Measurement of blood pressure by noninvasive (indirect) method & Measurement of blood pressure by invasive (direct) method.

As a result of measurement of blood pressure by noninvasive (indirect) method for the formulation, the rats were trained for at least one week until the BP was steadily recorded with minimal stress and restrain. The first cardiovascular parameters were discarded and mean of five or six subsequent measurements were recorded. Systolic blood pressure was measured weekly for four weeks by indirect non-invasive tail-cuff method using Power Lab.

Deoxycorticosterone acetate (DOCA) was an agent commonly used to induce hypertension in experimental animals. Administration of DOCA for 4 weeks in unilateral nephrectomized rats produced a significant elevation ($p<0.05$) in systolic blood pressure (SBP) as measured by tail cuff

method on II, III and IV week when compared to sham control rats. Unilateral nephrectomized rats which received formulation A (FT1) and B (FA1) for 4 weeks along with DOCA significantly ($p<0.05$) reduced SBP on III and IV week as compared with SBP of unilateral nephrectomized DOCA-salt hypertensive rats, thus implying an antihypertensive effect. However, chronic administration of formulation A (FT1) and B (FA1) in unilateral nephrectomized rats for 4 weeks did not alter SBP as compared to sham control.

As a result of measurement of blood pressure by invasive (direct) method, After completion of treatment schedule rats from each group were anesthetized with urethane (120mg/100gm). Femoral vein was cannulated with fine polyethylene catheter for administration of the drug. Tracheostomy was performed and blood pressure was recorded from left common carotid artery using pressure transducer by direct method on Chart data system. Heparinized saline (100 IU/ml) was filled in the transducer and in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, heart rate, basal blood pressure was recorded.

The heart rate, basal arterial blood pressure and pressor responses to noradrenaline (NA), adrenaline (Adr), phenylepherine (PE), serotonin (5-HT) and angiotensin II (Ang II) were significantly ($p<0.05$) increased in unilateral nephrectomized DOCA-salt hypertensive rats as compared to sham control rats. The heart rate, basal arterial blood pressure and pressor responses to NA, Adr, PE, 5-HT and Ang II were significantly ($p<0.05$) reduced in case of unilateral nephrectomized DOCA-salt hypertensive rats that received formulation A (FT1) and B (FA1) for 4 weeks as compared to unilateral nephrectomized DOCA-salt hypertensive rats. The heart rate, basal arterial blood pressure and pressor responses to NA, Adr, PE, 5-HT and Ang II were not altered in case of formulation A (FT1) and B (FA1) treated unilateral nephrectomized rats as compared to sham control rats.

7. Future Prospects

The field of transdermal technology has opened a new avenue to develop effective and efficient ways of delivering pulmonary arterial hypertension for the pediatric patents. Exploring various ways of administering the known compounds with the help of different types of transdermal patch may result in excellent therapeutic results as compared to drugs administered through conventional delivery systems. In Present Project, we evaluated the developed system by *ex vivo* and few in *in*

vivo studies, However, more exhaustive *in vivo* studies are required to prove the effectiveness of drug delivery system in the pediatric pulmonary arterial hypertension. Hence the studies related to the evaluation of pediatric patents to be done to prove its efficiency as well as safety of the developed system. Same delivery system can also be utilised for the treatment of other pediatric related diseases can also be attached with the surface of the drug carriers and prove its effectiveness in crossing of transdermal route.

8. Conflict of interest

No conflict of interest

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