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Comparative Prediction Of Plasma Concentration, Blood–Brain Barrier Penetration, Intestinal Absorption And Skin Diffusion Of Aceclofenac And Its Complex With Cow's Ghee In Swiss Albino Mice

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Abstract: The aim of present investigation was to compare plasma, brain tissue concentration, intestinal absorption and skin diffusion of Aceclofenac per se and its 1:1 w/w optimized complex (formulation) prepared with cow's ghee in Swiss albino mice. Aceclofenac per se and its complex prepared with cows ghee (studied in our previous work) in dose 300mg/Kg, were administered orally to Swiss albino male mice and comparison of concentration in plasma for first three hrs, obtained (by centrifuging of blood samples) and brain for first three hrs (by brain homogenization) as well as intestinal absorption (by cutting 1.75 cm segment of jejunum, filled with 5mg/0.5ml) and *in-vitro* skin permeability for 18 hrs (through 2 cm diameter excised epidermis (skin) of mice) were carried out and analyzed at UV spectrophotometer at λ_{max} 274 nm. Aceclofenac 1:1 w/w proportion optimized complex has found to more bioavailable in plasma, significantly ($p \leq 0.05$) increase its concentration in brain, more facilitation through G.I tract as well as profound increase in *in-vitro* skin diffusion (revealed from flux and permeability coefficient value) as compare to Aceclofenac per se in Swiss albino mice. Such findings provide further understanding for the possible therapeutic effects of Aceclofenac per se and Aceclofenac 1:1 w/w proportion complex in further pre-clinical and clinical research.

Keywords: Blood brain barrier; cow ghee; Aceclofenac; drug penetration; complex formation; brain homogenization, intestinal absorption, skin diffusion.

1. Introduction

Aceclofenac[1] – BCS *class* II drug, having anti-inflammatory, analgesic properties, used in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It works by blocking the action of a substance in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of prostaglandins (chemicals in the body) which cause pain, swelling and inflammation. Aceclofenac has oral bioavailability 60-70% after oral administration with protein binding 99% and $t_{1/2}$ approx. 4 hrs[2].

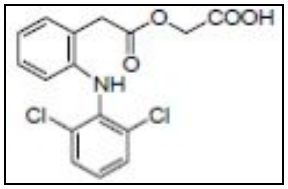

Fig. 1: Structure of Aceclofenac

Go-Ghrita (GG), Sanskrit Indian word is common name for cow ghee. GG, along with other substances, composed of numerous saturated fatty acids like myristic, stearic, lauric, butyric, capric, caprylic and unsaturated fatty acids like linoleic, linolenic, vaccenic and arachidonic acids[3], leads to difficulty in proposing any single chemical structure of it. Among these fatty acids, palmitic acid, a 16:0 saturated fatty acid, constitutes 29.95%, while oleic acid, which is 18: 1 monounsaturated acid with a double bond between 9-10 carbon atoms, is present to the extent of 27.42%[4].

In our previous work, we examined the evidence for complex formation of Aceclofenac with go-ghrita by FT-IR, Iin-vitro release, DSC, X-RD, NMR and SEM[5] using various sophisticated analytical techniques (FT - Infrared spectroscopy, *In-vitro* dissolution study, Differential scanning calorimetry, X-ray diffraction, Nuclear magnetic resonance and Scanning electron microscopy). In present investigation, attempt has been made to assess whether such sustenance release inclusion complexation with cow ghee can influence the rate of absorption of complexed drug through G.I. tract, plasma concentration and skin permeation as well as the passage of such prepared complexes through physiological barrier like blood brain barrier, since the complexes are more lipids soluble in nature, if their rate of absorption could be faster as compared to Aceclofenac per se in experimental animals (Swiss albino male mice).

2. Experimental

2.1. Apparatus

A UV –1700, Visible double beam spectrophotometer , Schimadzu®, Japan with 10 mm matched quartz cells was used for experiments operating at λ_{max} 274 nm.

2.2. Reagents and materials

Aceclofenac as a gift sample was kindly supplied by Zim Laboratories Ltd., Kalmeshwar, Nagpur. Go-ghrita was purchased from Magan Sangrahalay, Wardha, India. All other chemicals and reagents used were of analytical grade and were procured.

2.3. Animals study and drug treatments

2.3.1 Selection of experimental Animals

Healthy Swiss albino mice of either sex weighing 30-40g were used in this study. All the animals were obtained from Animal house, Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra. The animals were housed comfortably in a group of three, containing 9 mice in each group, excluding control group with 3 mice shown in table 1 in a single clean plastic cage with a metal frame lid on its top. They were housed under standard environmental conditions of temperature $(24\pm1)^0$C) and relative humidity of 30-70 %. A 12:12 hr light/dark cycle was followed. Before the test, animals were fasted prior to dosing by withholding food overnight, but not water. All the experimental procedures and protocols used in this study were reviewed and approved via the Approval No. CPCSEA/IAEC/CP_PL/24-2012 by the Institutional Animal Ethical Committee (IAEC), Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra (Regd. No. 729/02/a/CPCSEA) constituted in accordance with the guidelines of the CPCSEA, Government of India.

Table 1. Grouping and weighing of Swiss albino mice for animal study

Group	Weighing of Swiss albino mice (gram)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Avg. wt**
I	35	33	31	--	--	--	--	--	--	33
II	29	31	30	31	31	32	31	33	31	31
III	30	29	28	32	31	29	28	29	33	29.88

(Group-I indicates control, Group-II indicates Std. Aceclofenac and Group-III indicates Test optimized 1:1 Aceclofenac complex (formulation) prepared with cow ghee)

2.3.2 Preparation of drugs and chemical solutions

Oral dose (300 mg/kg) of Aceclofenac per se and formulation in suspension form were prepared by adding 1 ml of 1 % sodium carboxymethyl cellulose and one drop of tween 80.

2.4 Comparison of plasma concentration of Aceclofenac per se and formulation[6-10]:

Aceclofenac per se and its optimized complex 1:1 w/w with cow ghee were administered orally to mice. Blood samples (by retro-orbital method) were collected in epin drop tube with prior addition of drop of sodium heparin injection after 1hr, 2hr and 3hr of dosing in each group. The plasma was obtained by centrifuging of blood samples from respective treatment group and diluted with phosphate buffer pH 6.8 and analysed at UV spectrophotometer at λ_{max} 274 nm.

2.5 Comparison of concentration of Aceclofenac per se and formulation in brain of Swiss albino mice[6-10]:

The isolated brain was homogenized in ice chilled phosphate buffer (pH 6.8) and centrifuged. In supernatant solution, mixture of 0.5 M hydrochloric acid and hexane in 1:5 were added. The supernatant organic layer was separated by centrifugation and directly used to estimate the concentration of Aceclofenac per se and formulation by using UV spectrophotometer at λ_{max} 274 nm. Drug-free (i.e. blank) brain tissue was also obtained from the control mice which were injected with solvent alone. The concentration of Aceclofenac per se and formulation in brain was determined after 1hr, 2hr and 3hr of dosing in each group. Figure 2 showing the images of control, standard and test group while figure 3 shows feeding of distilled water to Swiss albino mice during study.

Figure 2. Grouping of Swiss albino mice as standard and test

Figure 3. Feeding of distilled water to Swiss control, albino mice

2.6 Comparison of intestinal absorption of Aceclofenac per se and formulation in Swiss albino mice[11]:

Mice weighing 30-40 g were anaesthetized with ether before the experiments. A 1.75 cm segment of jejunum was quickly removed, rinsed with normal saline and everted. This segment was tied at one end with a cotton thread, filled with Aceclofenac (5mg/0.5ml) solution and formulation (5mg/0.5ml), and then tied at the other end to aerator tube which was placed in organ bath containing phosphate buffer pH 6.8. The sampling were carried out after every 60 minutes to 3 Hrs. Withdrawal sample after dilution was directly used for analysis at UV spectrophotometer at λ_{max} 274 nm.

2.7 Comparison of absorption of Aceclofenac per se and formulation through excised epidermis (skin) of Swiss albino mice[12]:

For comparing absorption of Aceclofenac per se and formulation, *in-vitro* skin permeability study through excised epidermis (skin) of mice was carried out. Mice (30–35g) could have free access to food and water until used for the study. Each mice was anaesthetized with ether before the experiments. The dorsal hair was removed with a clipper and full thickness skin was surgically removed from each mice. The skin (2 cm in diameter) was tied to the Franz diffusion cell (receptor cell) such that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell. The whole assembly was fixed on a plate magnetic stirrer and stirred the solution in the receptor compartment constantly and continuously using magnetic beads and maintained the temperature at 37 ± 0.5^0C. Withdrawal of 1ml sample after interval of every 1 hr to 18 hrs from receptor compartment of Franz diffusion cell was carried out, after making dilution it was analyzed at UV spectrophotometer at λ_{max} 274 nm. % cumulative absorption of Aceclofenac and

formulation were calculated. The mean cumulative amount of drug permeated per unit surface area of the skin versus time was plotted and from the slope of linear portion of the plot the value of flux Jss (μg/cm^2/hr) was determined. The permeability coefficient was calculated using following equation.

$K_p = Jss.Cv$

Where, K_p is permeability coefficient and Cv is the total amount of drug.

2.8 Pharmacokinetic application and statistical analysis[13]:

The t-test was performed on all collected mean data obtained from animal studies. Significance was accepted at $p \leq 0.05$. Pharmacokinetic calculations were performed on each individual set of data using Graph prism pad 6 Demo version software by non-compartmental method. Pharmacokinetic results are represented as mean ± SEM. Statistical analysis was performed by t test to compare different groups. All experiments were carried out in triplicate with level of significance was set at $p < 0.05$.

3. Result and Discussion

3.1 Comparison of concentration of Aceclofenac per se and formulation in plasma and brain of Swiss albino mice

3.1.1 Comparison of plasma concentration of Aceclofenac per se and formulation[6-8]:

Plasma concentration of Aceclofenac per se and formulation was 214±0.12 μg/ml, 298.99±0.20 μg/ml in 1 hr; 156.6±0.32 μg/ml, 206.9±0.11 μg/ml in 2 hr; 104.44±0.17 μg/ml, 143.76±0.23 μg/ml in 3 hr respectively and summarized in table 2 and figure 6, showing P value in between 0.01 to 0.05 when one-way ANOVA test Newman-Keuls, Multiple Comparison Test. Figure 4 showing the oral administration of drug per se and formulation, while figure 5 indicate the blood withdrawal from Swiss albino mice by retro orbital method.

Figure 4. Oral administration of drug per se and its formulation to Swiss albino mice

Figure 5. Blood withdrawal from Swiss albino mice by retro-orbital method

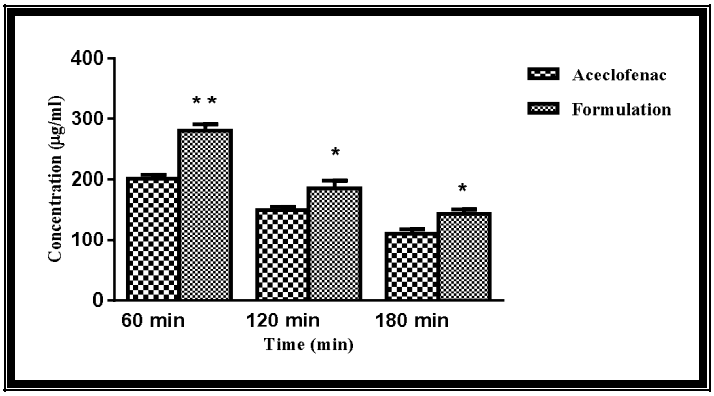

Figure 6. Plasma concentration of Aceclofenac and formulation in Swiss albino mice by retro orbital method (* represents P<0.05 whereas ** represents P<0.001)

Table 2. Plasma concentration of Aceclofenac and formulation in Swiss albino mice by retroorbital method

Time (Min)	Plasma concentration* (µg/ml)	
	Aceclofenac	Formulation
60	214.40±0.12	298.99± 0.20
120	156.60±0.32	206.90±0.11
180	104.44±0.17	143.76±0.23

*** Values indicates (Mean ± S. D.) when sample size (n=3)**

3.1.2 Comparison of concentration of Aceclofenac per se and formulation in brain of Swiss albino mice[9,10]:

Data obtained related to the concentration of Aceclofenac per se and formulation in brain was 10.12±0.19 µg/ml, 18.47±0.11 µg/ml in 1 hr; 6.05±0.27 µg/ml, 10.22±0.25 µg/ml in 2 hr; 4.11±0.09 µg/ml, 8.32±0.19 µg/ml in 3 hr respectively and were summarized in table 3 and figure 9 showing P value in between 0.01 to 0.05 when one-way ANOVA test Newman-Keuls, Multiple Comparison test was applied, whereas figure 7 and figure 8 reflecting isolated brain of Swiss albino mice and their images after isolation of Swiss albino mice brain.

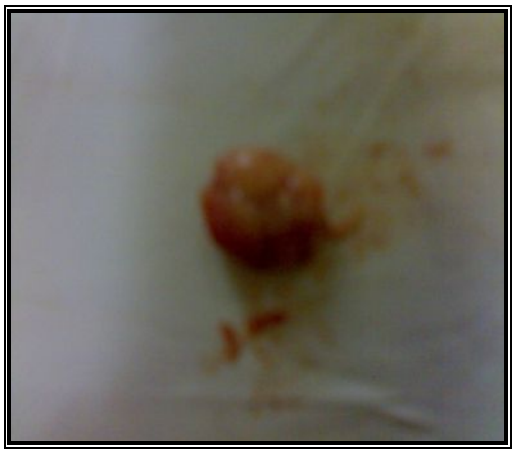

Figure 7. Isolated brain of Swiss albino mice

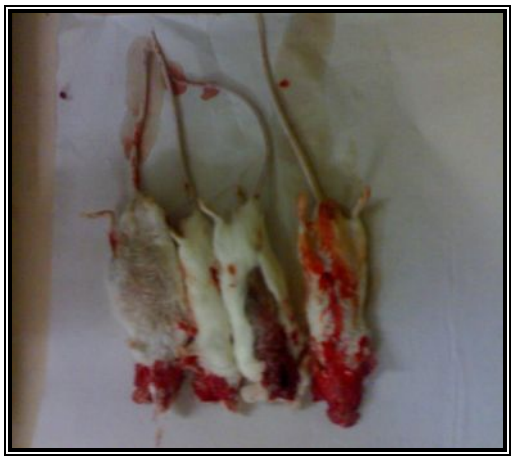

Figure 8. Swiss albino mice after isolation of brain

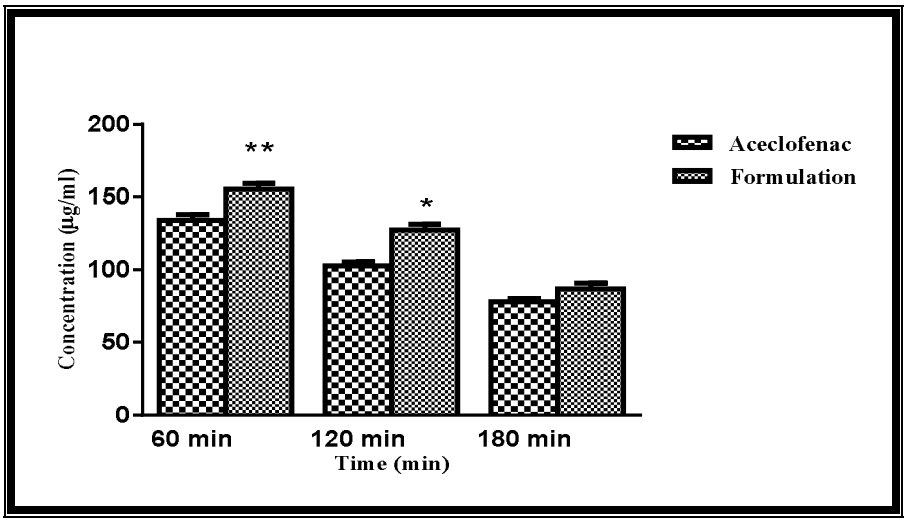

Figure 9. Brain concentration of Aceclofenac and formulation in Swiss albino mice by brain homogenization method (* represents P<0.05 whereas ** represents P<0.001)

Table 3. Brain concentration of Aceclofenac and formulation in Swiss albino mice by brain homogenization method

Time	Brain concentration* (µg/ml)	
(Min)	Aceclofenac	Formulation
60	10.12±0.19	18.47±0.11
120	6.05±0.27	10.22±0.25
180	4.11±0.09	8.32±0.19

* Values indicates (Mean ± S. D.) when sample size (n=3)

3.2 To examine and compare intestinal absorption of Aceclofenac per se and formulation in Swiss albino mice[11]:

Figure 10 depicted assembly set up for this study. Intestinal absorption data obtained from segment of jejunum after filling solution of Aceclofenac per se and formulation was 122.10±0.37 µg/ml, 177.83±0.11 µg/ml in 1 hr; 92.11±0.49 µg/ml, 107.65±0.24 µg/ml in 2 hr; 42.33±0.23 µg/ml, 88.45±0.14 µg/ml in 3 hr respectively and were summarized in table 4 where as P value obtained from figure 12 was in between 0.01 to 0.05 when one-way ANOVA test Newman-Keuls, Multiple Comparison test showing significance.

Figure 10. Organ bath assembly set up for intestinal absorption of drug

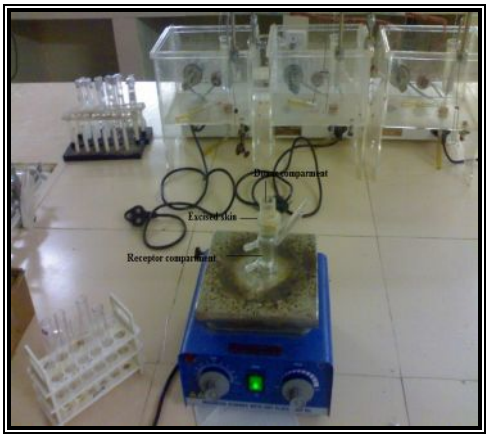

Figure 11. In-vitro drug absorption study by Franz – type diffusion cell

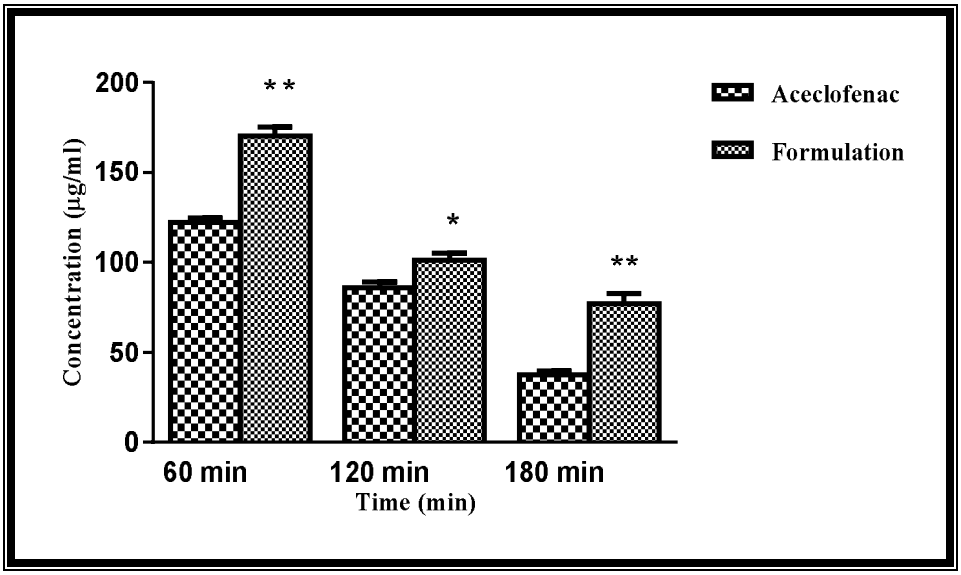

Figure 12. Intestinal absorption of Aceclofenac and formulation in Swiss albino mice (* represents P<0.05 whereas ** represents P<0.001)

Table 4. Intestinal absorption of Aceclofenac and formulation in Swiss albino mice

Time	Intestinal absorption* (µg/ml)	
(Min)	Aceclofenac	Formulation
60	122.10±0.37	177.83±0.11
120	92.11±0.49	107.65±0.24
180	42.33±0.23	88.45±0.14

* Values indicates (Mean ± S. D.) when sample size (n=3)

3.3 To examine and compare absorption of Aceclofenac per se and formulation through excised epidermis (skin) of Swiss albino mice[12]:

Figure 11 demonstrated the assembly set up for *in-vitro* drug absorption study by Franz – type diffusion cell, % cumulative absorption of Aceclofenac per se and formulation showed 41.22±0.15 and 88.29±0.19 respectively through hairless excised epidermis (skin) of Swiss albino mice into receptor compartment within 18 hrs as shown in table 5 and figure 13 – figure 14. For determination of Flux value i.e Jss (µg/cm^2/hr), data were plotted as amount of Aceclofenac permeated Vs time in hr (figure 15) and amount of formulation permeated Vs time in hr (figure 16), summarized in table 6. Permeability coefficient and Flux value was calculated using formula for Aceclofenac per se and formulation and was found to 0.0058 cm/hr, 58.01 µg/cm^2/hr and 0.0117 cm/hr, 117.80 µg/cm^2/hr respectively, summarized in table 7. Radius of the excised epidermis (skin) of Swiss albino mice taken for the study = 1 cm and Area of the skin = $\pi r^2 = 3.14\ (1)^2 = 3.14$.

P value obtained from figure 5.36 was in between 0.01 to 0.05 when one-way ANOVA test Newman-Keuls, Multiple Comparison Test showing significance.

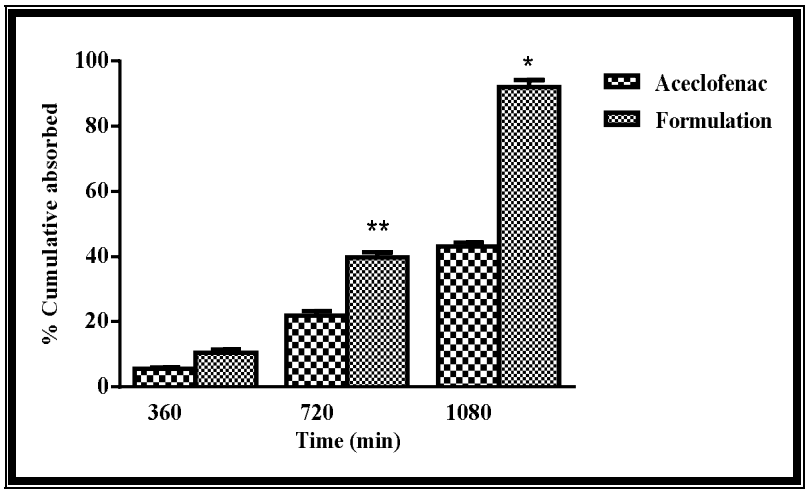

Figure 13. *In vitro* skin permeability study of Aceclofenac and formulation through excised epidermis (skin) of Swiss albino mice (* represents P<0.05 whereas ** represents P<0.001)

Table 5. *In vitro* skin permeability study of Aceclofenac and formulation through excised epidermis (skin) of Swiss albino mice

Time	% cumulative Aceclofenac absorbed*	
(Min)	Aceclofenac	Formulation
60	0.22±0.04	0.60±0.09
120	0.69±0.02	1.60±0.11
180	1.39±0.08	2.88±0.12
240	2.35±0.21	4.50±0.45
300	3.47±0.33	6.36±0.64
360	4.89±0.23	8.86±0.55
420	6.63±0.34	12.16±0.51
480	8.61±0.44	16.03±0.63
540	10.92±0.55	20.54±0.24
600	13.43±0.12	25.77±0.43
660	16.21±0.19	31.65±0.18
720	19.22±0.27	37.72±0.21
780	22.49±0.29	44.11±0.25
840	26.14±0.31	51.08±0.29
900	29.89±0.54	60.07±0.61
960	33.64±0.45	69.46±0.43
1020	37.43±0.31	78.87±0.56
1080	41.22±0.15	88.29±0.19

* Values indicates (Mean ± S. D.) when sample size (n=3)

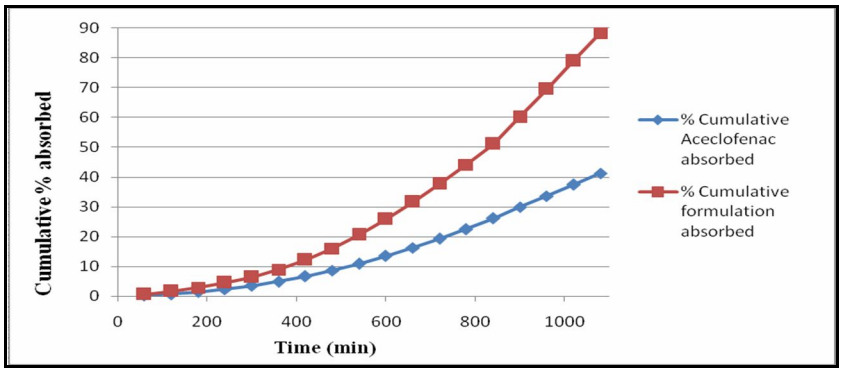

Figure 14. Cumulative % absorption of Aceclofenac and formulation

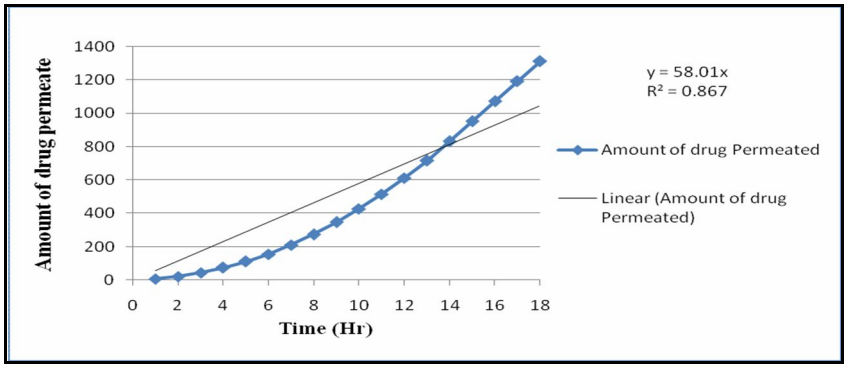

Figure 15. Amount of Aceclofenac permeate Vs time profile for flux calculation

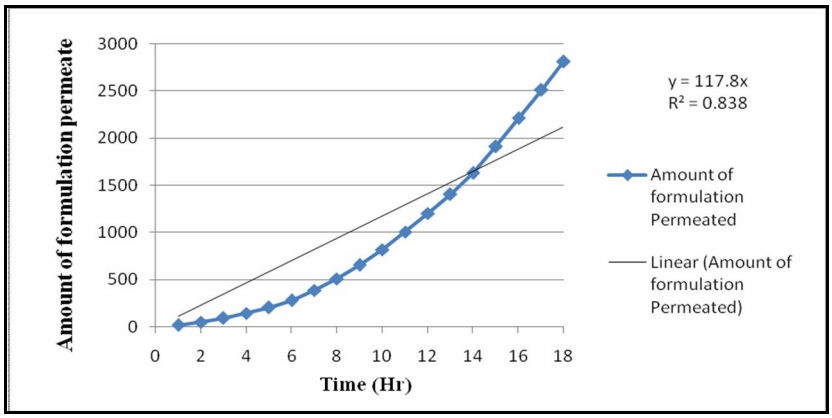

Figure 16. Amount of formulation permeate Vs time profile for flux calculation

Table 6. Amount of Aceclofenac and formulation permeated through excised epidermis (skin) of Swiss albino mice

Time	Amount permeated (µg/cm^2)	
(Hr)	Aceclofenac	Formulation
1	7.01	19.24
2	21.97	50.83
3	44.27	91.85
4	74.84	143.18
5	110.51	202.68
6	155.73	282.17
7	211.15	387.39
8	274.20	510.45
9	347.77	654.14
10	427.71	820.76
11	516.24	1007.9
12	612.10	1201.15
13	716.24	1404.71
14	832.48	1626.75
15	951.91	1913.12
16	1071.34	2211.98
17	1192.04	2511.85
18	1312.74	2811.98

Table 7. Flux values and Permeability coefficient of Aceclofenac and formulation from the excised epidermis (skin) of Swiss albino mice

Parameters	Aceclofenac	Formulation
Flux (µg/cm^2/hr)	58.01	117.80
Permeability coefficient (cm/hr)	0.0058	0.0117

4. Conclusion

Present investigation, predicted plasma and brain concentration as well as intestinal absorption and skin diffusion of Aceclofenac and formulation in Swiss albino mice. It is noteworthy of mentioning that formulation has found to more bioavailable in plasma when the blood is withdrawal by retro-orbital method and tested for first three hours; significantly increase its concentration in brain when estimated by brain homogenization technique, more facilitation through G.I tract (segment of jejunum) for first three hours as well as profound

increase *in-vitro* skin (excised epidermis) diffusion for eighteen hours, revealed from Permeability coefficient and Flux values as compare to Aceclofenac per se in Swiss albino mice with significance ($p \leq 0.05$). Such findings provide further understanding for the possible therapeutic effects of Aceclofenac per se and Aceclofenac 1:1 w/w proportion complex in further pre-clinical and clinical research.

5. Acknowledgements

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