ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2016; 7(2): 14 - 21

RESEARCH ARTICLE

Comparative Prediction of Plasma Concentration, Blood Brain Barrier Penetration, Intestinal Absorption and Skin Diffusion of Diclofenac Sodium and its Complex with Cow's Ghee in Swiss Albino Mice

Bawankar R D[*1], Sakarkar D M[2], Dorle A K[3]

[1]*Research scholar, SudhakarraoNaik Institute of Pharmacy, Nagpur Road, Pusad – 445204 (M.S), India*
[2]*Principal, SudhakarraoNaik Institute of Pharmacy, Nagpur Road, Pusad – 445204, Dist. Yavatmal (M.S), India*
[3]*Former Professor, Emeritus & Ex- Head, Department of Pharmaceutical Sciences, RTM Nagpur University, (M.S), India*

Received 03Jan 2015; Revised 08Apr 2016; Accepted 19 Apr 2016

ABSTRACT

The aim and objective of present investigation was to compareplasma, brain tissue concentration, intestinal absorption and skin diffusion of Diclofenac sodium per se and its 1:1 w/w optimized complex (formulation)prepared with cow's ghee in Swiss albino mice. Diclofenac sodiumper se and its complex prepared with cows ghee (studied in our previous work) in dose 300mg/Kg, were administered orallyto Swiss albino male mice and comparison of concentrationin plasma for first three hrs, obtained (by centrifuging of blood samples) and brainfor 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 permeabilityfor 18 hrs(through 2 cm diameter excised epidermis (skin) of mice) werecarried out and analyzed at UV spectrophotometer at λ_{max}278 nm. Diclofenac sodium 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 Diclofenac sodium per se in Swiss albino mice.Such findings provide further understanding for the possible therapeutic effects of Diclofenac sodium per se and Diclofenac sodium 1:1 w/w proportion complex in further pre-clinical and clinical research.

Key words: Blood brain barrier; cow ghee; Diclofenac sodium; drug penetration; complex formation; brain homogenization, intestinal absorption, skin diffusion.

1. INTRODUCTION

Diclofenac sodium[1]– BCS *class* II drug,is a non-steroidal anti-inflammatory drug (NSAID) advocated for use in painful and inflammatory rheumatic and certain non-rheumatic conditions. It is available in a number of administration forms which can be given orally, rectally or intramuscularly. It inhibits the enzyme, cyclooxygenase(COX), an early component of the arachidonic acid cascade, resulting in the reduced formation of prostaglandins, thromboxanes and prostacylin. Chemically, Diclofenac sodium is 2-[(2,6-dichlorophenyl)amino] benzene acetic acid. Diclofenac sodium has bioavailability 50 - 60% after oral administration with protein binding 99% and $t_{1/2}$ approx. 2 hrs[2].

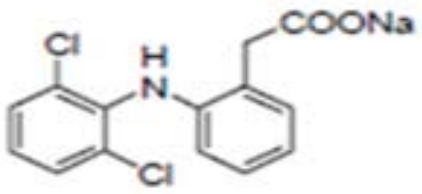

Fig 1:Structure of Diclofenac sodium

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

***Corresponding Author:**Ram D. Bawankar, **Email:** rambawankar2008@rediff.com.**Phone No:**9960820852

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 confirmatory evidence other than ^{1}HNMR spectroscopic technique for complex formation of Diclofenac sodium with go-ghrita[5]using various sophisticated analytical techniques (FT - Infrared spectroscopy, *In-vitro* dissolution study, Differential scanning calorimetric, X-ray diffraction and Scanning electron microscopy). In present investigation, attempt has been made to assess whether such sustenance release inclusion complexationwith 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 Diclofenac sodium per sein experimental animals(Swiss albino male mice).

2. EXPERIMENTAL

2.1. Apparatus

A UV –1700, Visible double beam spectrophotometer,Schimadzu®, Japan with 10 mm matchedquartzcells was used for experiments operating at λ_{max}278 nm.

2.2. Reagents and materials

Diclofenac sodium as a gift sample was kindly supplied by Zim Laboratories Ltd., Kalmeshwar, Nagpur. Go-ghrita was purchased from MaganSangrahalay, 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, SudhakarraoNaik 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 1in 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 hrlight/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, SudhakarraoNaik 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. Diclofenac sodium and Group-III indicates Test optimized 1:1 Diclofenac sodium complex (formulation) prepared with cow ghee)

2.3.2 Preparation of drugs and chemical solutions

Oral dose (300 mg/kg) of Diclofenac sodium 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 Diclofenac sodium per se and formulation[6-10]:

Diclofenac sodium 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 analysedat UV spectrophotometer at λ_{max}278 nm.

2.5 Comparison of concentration of Diclofenac sodium 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 Diclofenac sodium per se and formulation by using UV spectrophotometer at λ_{max}278 nm. Drug-free (i.e. blank) brain tissue was also obtained from the control mice which were injected with solvent alone. The concentration of Diclofenac sodium 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.

Fig 2. Grouping of Swiss albino mice asstandard and test

Fig 3.Feeding of distilled water to Swisscontrol,albino mice

2.6 Comparison of intestinal absorption of Diclofenac sodium per se and formulationin 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 Diclofenac sodium (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}278 nm.

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

For comparing absorption of Diclofenac sodium 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}278 nm. % cumulative absorption of Diclofenac sodium 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 ($\mu g/cm^2/hr$) 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 ≤ 0.05. p Pharmacokinetic calculations were performed on each individual set of data using Graph prism pad 6 Demo version softwareby 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. RESULTS AND DISCUSSION

3.1 Comparison of concentration of Diclofenac sodium per se and formulation in plasma and brain of Swiss albino mice:

3.1.1 Comparison of plasma concentration of Diclofenac sodium per se and formulation[6-8]: Plasma concentration of Diclofenac sodium per se and formulation was 231.11±0.35µg/ml, 342.90±0.41µg/ml in 1 hr; 156.70±0.17µg/ml, 177.60±0.32µg/ml in 2 hr; 94.60±0.34µg/ml, 127.88±0.29µg/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.

Fig 4: Oral administration of drug per sec and its formulation to Swiss albino mice

Fig 5: Blood withdrawal from Swiss albino mice by retro-orbital method

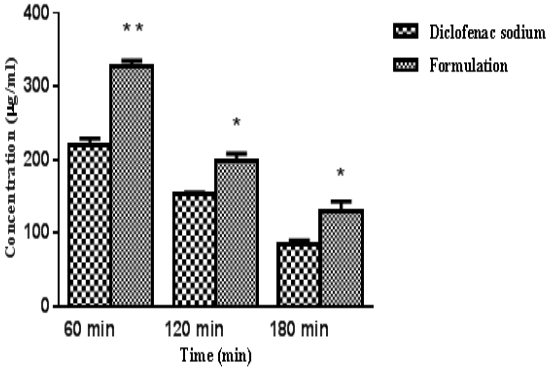

Fig6: Plasma concentration of Diclofenac sodium and formulation in Swiss albino mice by retro orbital method (* represents P<0.05 whereas ** represents P<0.001)

Table 2: Plasma concentration of Diclofenac sodium and formulation in Swiss albino mice by retroorbital method

Time (Min)	Plasma concentration* (µg/ml)	
	Diclofenac sodium	Formulation
60	231.11±0.35	342.90±0.41
120	156.70±0.17	177.60±0.32
180	94.60±0.34	127.88±0.29

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

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

Data obtained related to the concentration of Diclofenac sodium per se and formulation in brain was 11.09±0.25 µg/ml, 18.97±0.41 µg/ml in 1 hr; 7.02±0.31 µg/ml, 11.06±0.33 µg/ml in 2 hr; 4.71±0.29 µg/ml, 10.30±0.17 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&8**) reflecting isolated brain of Swiss albino mice and their images after isolation of Swiss albino mice brain.

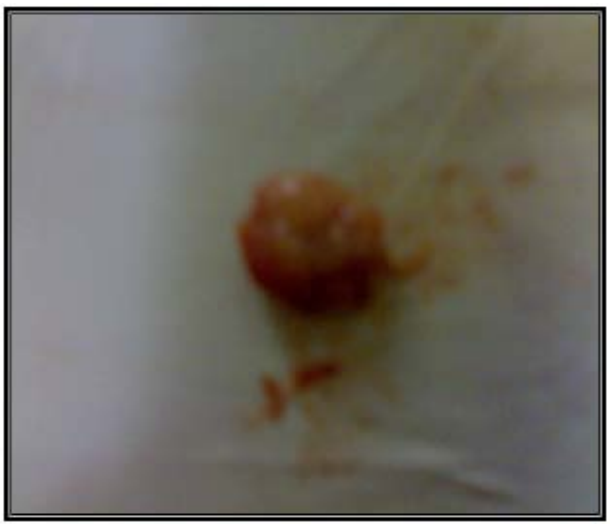

Fig 7: Isolated brain of Swiss albino mice

Fig 8: Swiss albino mice after isolation of brain

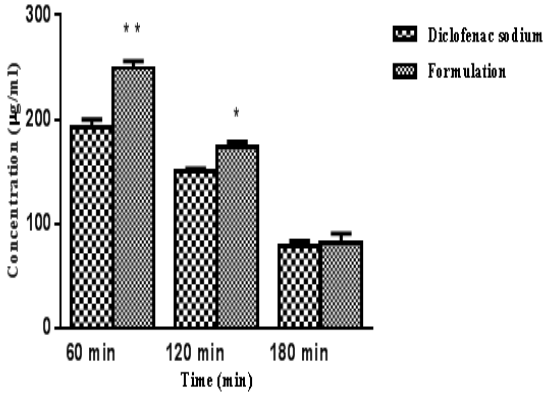

Fig 9:Brain concentration of Diclofenac sodium and formulation in Swiss albino mice by brain homogenization method(* represents P<0.05 whereas ** represents P<0.001)

Table 3: Brain concentration of Diclofenac sodium and formulation in Swiss albino mice by brain homogenization method

Time (Min)	Brain concentration* (µg/ml)	
	Diclofenac sodium	Formulation
60	11.09±0.25	18.97±0.41
120	7.02±0.31	11.60±0.33
180	4.71±0.29	10.03±0.17

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

3.2 To examine and compare intestinal absorption of Diclofenac sodium per se and formulationin 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 Diclofenac sodium per se and formulation was 134.99±0.34 µg/ml, 192.11±0.25 µg/ml in 1 hr; 121.11±0.11 µg/ml, 176.98±0.37 µg/ml in 2 hr; 56.44±0.25 µg/ml, 78.87±0.29 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

Fig 10: Organ bath assembly set up for intestinal absorption of drug

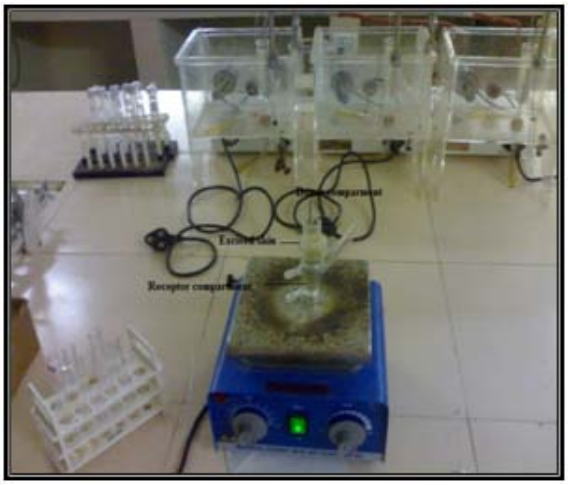

Fig 11: In-vitro drug absorption study by Franz – type diffusion cell

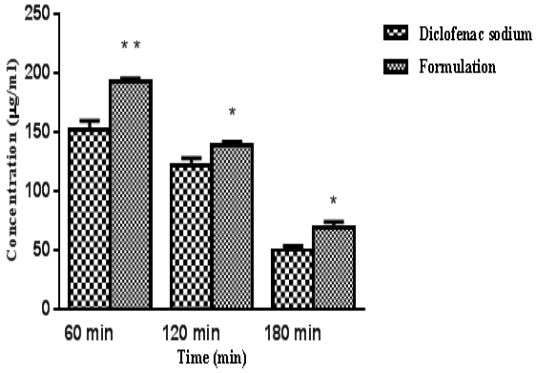

Fig 12: Intestinal absorption of Diclofenac sodium and formulation in Swiss albino mice (* represents P<0.05 whereas ** represents P<0.001)

Table 4: Intestinal absorption of Diclofenac sodium and formulation in Swiss albino mice

Time (Min)	Intestinal absorption* (µg/ml)	
	Diclofenac sodium	Formulation
60	134.99±0.34	192.11±0.25
120	121.10±0.11	176.98±0.37
180	56.44±0.25	78.87±0.29

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

3.3 To examine and compare absorption of Diclofenac sodium 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 Diclofenac sodium per se and formulation showed 40.63±0.31 and 86.42±0.27 respectively through hairless excised epidermis (skin) of Swiss albino mice into receptor compartment within 18 hrs as shown in (**Table 5 &Figure 13,14**). For determination of Flux value i.eJss (µg/cm^2/hr), data were plotted as amount of Diclofenac sodium 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 Diclofenac sodium per se and formulation and was found to 0.0057 cm/hr, 57.29 µg/cm^2/hr and 0.0115 cm/hr, 114.90µg/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.

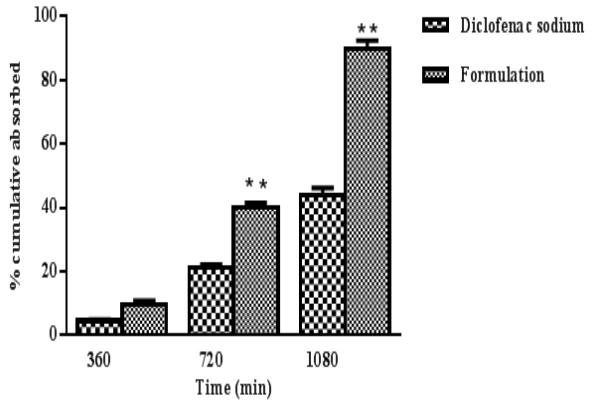

Figure 13:*In vitro* skin permeability study of Diclofenac sodium and formulation through excised epidermis (skin) of Swiss albino mice (* represents P<0.05 whereas ** represents P<0.001)

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

Time (Min)	% cumulative Diclofenac sodium absorbed*	
	Diclofenac sodium	Formulation
60	0.23 ±0.02	0.58 ±0.07
120	0.72 ±0.05	1.48 ±0.08
180	1.40±0.09	2.72 ±0.14
240	2.34±0.24	4.21 ±0.19
300	3.37±0.29	5.97 ±0.61
360	4.85±0.33	8.38 ±0.45
420	6.61±0.21	11.57 ±0.91
480	8.55 ±0.19	15.21 ±0.78
540	10.84 ±0.44	19.71 ±0.12
600	13.32 ±0.21	24.92 ±0.70
660	16.07 ±1.01	30.82 ±0.67
720	19.09 ±0.23	36.93 ±0.32
780	22.24 ±0.38	43.23 ±0.89
840	25.79 ±0.27	50.08 ±0.11
900	29.46 ±0.22	58.54 ±0.43
960	33.17 ±1.09	67.79 ±0.99
1020	36.90 ±0.21	77.10 ±0.33
1080	40.63 ±0.52	86.42 ±0.41

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

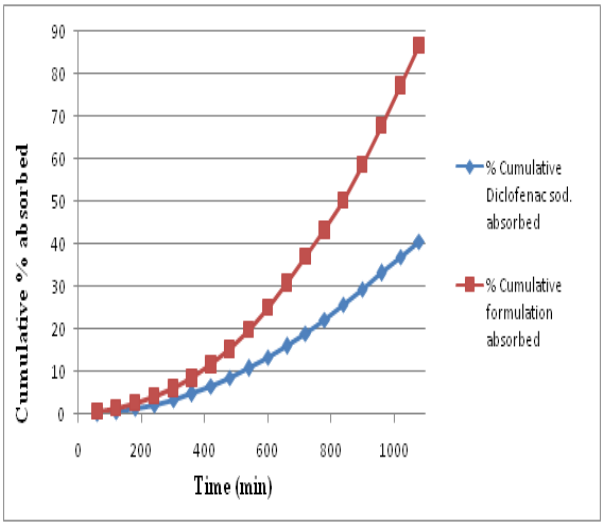

Fig 14:Cumulative % absorption of Diclofenac sodium and formulation

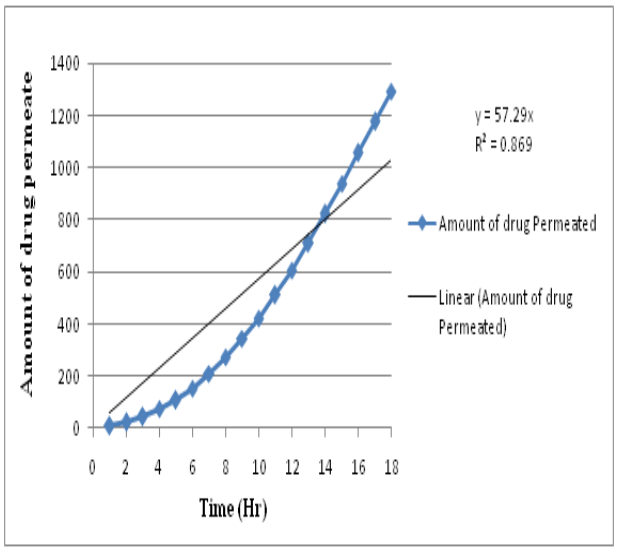

Fig 15:Amount of Diclofenac sodiumpermeate Vs time profile for flux calculation

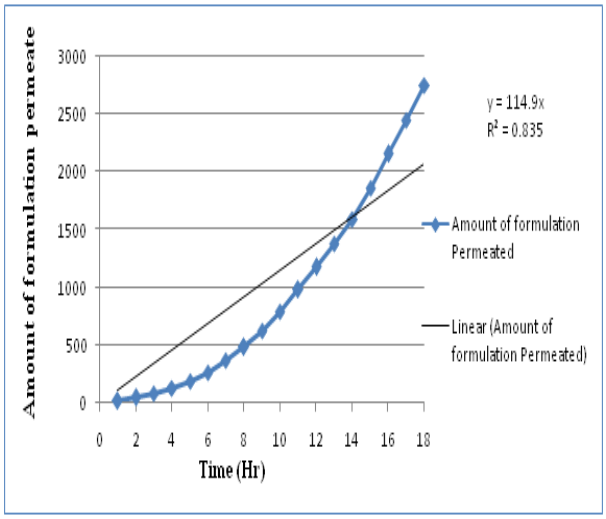

Fig 16:Amount of formulation permeate Vs time profile for flux calculation

Table 6: Amount ofDiclofenac sodium and formulation permeated through excised epidermis (skin) of Swiss albino mice

Time (Hr)	Amount permeated (µg/cm^2)	
	Diclofenac sodium	Formulation
1	7.32	18.50
2	22.93	46.94
3	44.59	86.56
4	74.52	134.08
5	107.32	190.03
6	154.46	266.94
7	210.51	368.47
8	272.29	484.49
9	345.22	627.77
10	424.20	793.47
11	511.78	981.54
12	607.96	1176.02
13	708.28	1376.69
14	821.34	1595.00
15	938.22	1864.20
16	1056.37	2158.89
17	1175.16	2455.32
18	1293.95	2752.23

Table 7: Flux values and Permeability coefficient of Diclofenac sodium and formulation from the excised epidermis (skin) of Swiss albino mice

Parameters	Diclofenac sodium	Formulation
Flux (µg/cm^2/hr)	57.29	114.90
	0.0057	0.0115

4. CONCLUSION

Present investigation, predicted plasma and brain concentration as well as intestinal absorption and skin diffusion of Diclofenac sodium 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 Diclofenac sodium per se in Swiss albino mice with significance ($p \leq 0.05$). Such findings provide further understanding for the possible therapeutic effects of Diclofenac sodium per se and Diclofenac sodium 1:1 w/w proportion complex in further pre-clinical and clinical research.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the useful discussion with Dr. S. V. Tembhurne, H.O.D, Department of Pharmacology, SudhakarraoNaik Institute of Pharmacy, Pusad, (M.S.), India. In addition, the authors would like to thank Mr. MangeshWandhare, Research Scholar, SudhakarraoNaik Institute of Pharmacy, Pusad, (M.S.), Indiafor the support and help during work.

REFERENCES

1. Baldessarini RJ., Goodman and Gilman's: The pharmacological basis of Therapeutics. 11th edition. McGraw-HILL Medical Publishing Division, 2007. p. 443.
2. Barar FSK. Essentials of Pharmacotherapeutics. S. Chand and Company Ltd., New Delhi. 2003; p. 128.
3. Rangappa KS and Achaya KT: Indian Dairy Products, Bombay. Asia Publishing House. 1974; 1- 386.
4. Ramamurthy MK and Narayanan KM: Fatty acid compositions of buffalo and cow milk fats by GLC. Milchwissenschaft. 1971; 26: 693-697.
5. Bawankar RD., SakarkarDM.,Dorle AK. Confirmatory evidence other than ^{1}HNMR spectroscopic technique for complex formationofDiclofenac sodium with go-ghrita. International Journal of Bioassays. 2014; 3(9): 3218 – 3224.
6. Masaya F., Kiyoyuki K., Fumie A., Yohei F., Kenji T., Kenzo T., Tsuneo M., Takaaki H. Altered brain penetration of Diclofenac and Mefenamic acid, but not Acetaminophen, in shiga-like toxin ii-treated mice. J Pharmacol Sci. 2005; 97: 525 – 532.
7. Tsai YM., Chien CF., Lin LC., Tsai TH. Curcumin and its nano-formulation: The kinetics of tissue distribution and blood–

brain barrier penetration. Int J Pharm. 2011; 15; 416(1): 331-8.
8. Poetsch V., Jiran K., Simon K., Stimpfl T. Determination of drugs in brain samples using disposal pipette extraction. ToxichemKrimtech. 2011; 78: 340 – 343.
9. Kondawar MS., Shinde SR. Development and validation of bioanalytical method for lacosamide from rat brain tissues. International Journal of Pharm Tech Research. 2012; 4(4): 1417 – 1421.
10. Lundquist S., Renftel M., Brillault J., Fenart L., Cecchelli R., Dehouck MP. Prediction of drug transport through the blood-brain barrier in vivo: A comparison between two *invitro*cell models. Pharmaceutical Research. 2002; 19(7): 977-981.
11. Tembhurne SV., Sakarkar DM. Transport of glucose in isolated goat ileum preparation: An alternative research tool for absorption study. Indian J PhysiolPharmacol. 2008; 52(2): 211–213.
12. Fern S., Rouse J., Sanderson D., Eccleston G. A comparative study of transmembrane diffusion and permeation of Ibuprofen across synthetic membranes using franz diffusion cells. Pharmaceutics. 2010; 2: 209 – 223.
13. Townend J. Practical statistics for environmental and biological scientists. England, John Wiley and Sons Ltd. 2002, 270-276.