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PREPARATION OF ACECLOFENAC PRODRUGS, *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT

The aim of this study was to synthesize prodrugs of aceclofenac, chemically is a phenyl acetic acid derivative used as NSAID (Non Steroidal Anti Inflammatory Drug) for treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. The main adverse effect, associated with the drug is upper G.I tract complications and has to be used along with an H₂ antagonist to overcome the adverse effect. Hence, prodrugs of aceclofenac were prepared by using PEG 1500 (polyethylene glycol) and PEG 6000 as polymeric backbone and a linker glycine an aminoacid. The prodrugs prepared were characterized by FT-I.R and N.M.R. *In vitro* dissolution study revealed the drug release from the prodrugs, was higher at pH 7.2 than at pH 1.2. *In vivo* evaluation studies of the prodrugs, for anti inflammatory activity, and ulcer protection activity, all the prodrugs retained their activity found to be more protective towards ulcers than the standard drug.

KEY WORDS: Aceclofenac, prodrugs, polyethylene glycol, ulcer protecting activity, anti inflammatory.



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INTRODUCTION

Inflammation is a host defence mechanism, which involves cell-cell, cell-mediator and tissue interaction¹. The mechanism of NSAID's is by inhibiting COX-2 selectively which are required for production of prostaglandins². Aceclofenac is a phenyl acetic acid derivative and chemically is [2-[2-(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid^{3, 4, 5, 6} used as NSAID. It is used for its anti inflammatory activity, osteo arthritis, rheumatoid arthritis and ankylosing spondylitis and a maximum dose of 200 mg per day is given, 100 mg in case of renal impaired patients³. The adverse effects associated with the drug include upper GI tract irritation⁷.

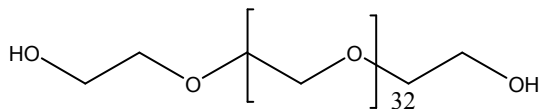
Prodrugs

These are inactive as such, but converted to active drugs in the body⁸. Prodrug approach is a type of chemical delivery system involving chemical modification of drug⁹. For prodrug approach polymers were used as carriers, include PEG (polyethylene glycol)¹⁰, PVP (poly vinyl pyrrolidone) as backbone¹¹. Prof H. Ringsdorf first named polymeric prodrugs using polymer as backbone¹². Some polymeric prodrugs synthesized include PEG-acyclovir¹³, aspirin with hydroxyl ethyl methacrylate¹⁴ (HEMA). Polyethylene glycols¹⁵ of different molecular weights were most commonly used as polymeric backbone¹⁶ for synthesis of prodrugs¹⁷. Linking of a drug to PEG through covalent bonding is termed as PEGylation¹⁸. The drugs can be bonded to PEG through carbonate, anhydride, ester, amide linkages to produce polymer-drug conjugates¹⁹. Solid dispersion of

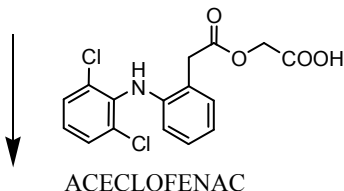
aceclofenac was prepared using PEG 6000, PVP²⁰. Size enlargement of aceclofenac was done by melt granulation and liquisolid technique and found increased solubility and dissolution²¹. Mouth dissolving tablets of aceclofenac were developed by using solid dispersion technique using PEG 6000 as carrier²². The dissolution rate of aceclofenac was increased by using PEG 6000 as carrier in different ratios on solid dispersions²³. To overcome the adverse effects of NSAID's, a prodrug approach has been selected²⁴. Aceclofenac prodrugs were prepared using PEG 1500 and PEG 6000 by esterification and along with linker by amide linkage. The prepared prodrugs were characterized by FT-I.R and N.M.R. The drug conjugates were subjected to dissolution studies for the drug release. *In vivo* anti inflammatory activity and ulcer protecting activity was performed for the prodrugs.

MATERIALS AND METHODS

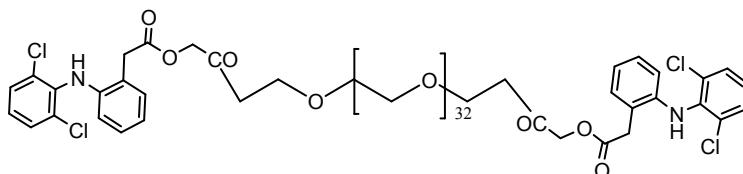
Aceclofenac drug was obtained from Hetero Drugs, Hyderabad. PEG 1500, PEG 6000, DMF (Di methyl formamide), DCC (Dicyclo hexyl carbodiimide), DMAP (Dimethyl amino pyridine), Glycine and other solvents of reagent grade were purchased from SD Fine chemicals. Animal studies were conducted in the department of pharmacology, Geethanjali College of Pharmacy, Keesara, Ghatkesar and approved by Institutional Animal Ethics Committee, Regd no: 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013 for anti inflammatory and ulcer protecting activity.



POLY ETHYLENE GLYCOL 1500/6000



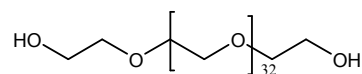
ACECLOFENAC



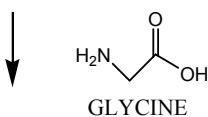
POLY ETHYLENE GLYCOL1500/6000- ACECLOFENAC

Preparation of PEG 1500/6000-Aceclofenac

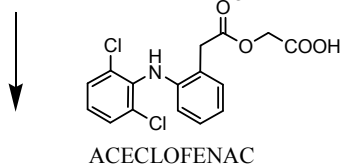
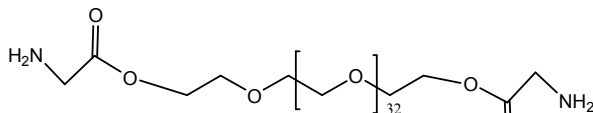
PEG 1500/6000 1.5gms and 1.6 ml of pyridine were taken in a round bottom flask, to it a solution of DCC 1gm and 0.6 gms of DMAP in 10 ml DMF was added. The flask was kept in an ice bath and temperature was maintained 0° C, to this aceclofenac was added for 10 mints. The flask was then placed on a magnetic stirrer, attached with a condenser and was allowed for coupling reaction for 7 days at room temperature. The residue obtained was dissolved in DCM (dichloro methane) and reprecipitated by excess of cold diethyl ether²⁵. The product obtained was confirmed by TLC (Thin Layer Chromatography) using mobile phase DCM : methanol 3:2. Finally, confirmed by FT-I.R and N.M.R.



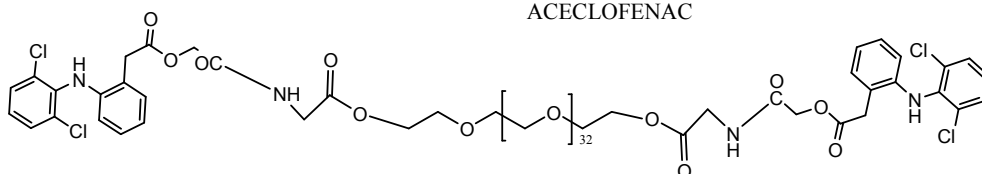
POLY ETHYLENE GLYCOL1500/6000



GLYCINE



ACECLOFENAC



POLY ETHYLENE GLYCOL1500/6000-GLYCINE-ACECLOFENAC

Preparation of PEG 1500/6000-Glycine

1500/6000 1.5 gms and 1.6 ml of pyridine were taken in a two necked round bottom flask and 20 ml of DMF was added and placed on a magnetic stirrer. To this 0.18 gms of glycine was added in small portions for 3 hrs maintaining at room temperature. Then the contents were refluxed by attaching a condenser, maintaining a temperature of 130° C for 21 hrs. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The formation of the product was confirmed by TLC and mobile phase DCM: methanol 3:2 and structures were confirmed by FT-I.R and N.M.R.

Preparation of PEG 1500/6000-glycine-Aceclofenac

A solution of DCC 1 gm in 10 ml DMF and 0.6 gms of DMAP in 10 ml of DMF was taken in a beaker. The mixture was added drop by drop to another beaker containing a solution of 0.4 gms of PEG 1500/6000-glycine in 20 ml of DMF. Aceclofenac 1 gm was added in portions to the above mixture at 0° C for 10 mints. The contents were transferred to a round bottom flask fitted with a condenser and placed on magnetic stirrer. The coupling reaction was carried out for 7 days at room temperature. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The product obtained was confirmed by TLC and mobile phase DCM : Methanol 3:2 and structures were confirmed by FT-I.R and N.M.R.

In vitro Drug Release studies

The prodrugs prepared were subjected to dissolution studies at pH 1.2 and 7.2, temperature was maintained at 37° C. The λ_{max} was determined for aceclofenac and found to be 275 nm, aliquots of 5 ml were collected at intervals of 0, 5, 10, 15, 30, 45, 60 mints, sink conditions were maintained. A

standard graph was plotted and % drug release was found. A graph of time v/s cumulative drug release was plotted at pH 1.2 and 7.2.

Anti inflammatory Activity

Male Sprague-Dawley rats weighing 100-150 gms were divided into 6 groups. The method for anti inflammatory activity followed was Carrageenan induced Rat Paw Edema. Ist group control, IInd group standard drug, IIIrd, IVth, Vth and VIth groups received aceclofenac prodrugs, injected in subplantar region in the left and right hind paws and the swelled volume was measured by Dolphin, India Plethysmometer. The measurements were taken at intervals of 1, 3, 6 hrs^{26, 27}. The % inhibition was calculated by formula

$$\% \text{ Inhibition} = \frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{test}}}{(V_t - V_0)_{\text{control}}} \times 100$$

Ulcer protecting Activity

For ulcer protecting activity Pylorus-ligation method was followed. The animals weighing 100-150 gms were fastened overnight, anaesthetized, incised 1 cm long in abdomen below the sternum. The stomach was exposed and a thread was passed round pyloric sphincter, a knot was tied. Abdomen was closed with sutures and animals were kept in a separate cage, allowed to recover. Ist group control, IInd group received standard drug aceclofenac 10 mg/kg, IIIrd, IVth, Vth, VIth groups were injected prodrugs of aceclofenac and after performing pylorus ligation kept in separate cages, after 4 hrs the animals were sacrificed and abdomen was cut open, stomach was removed and washed under running tap water, then placed on glass slide and observed on microscope at 10 X magnification for ulcers²⁸.

Ulcer Index was calculated by

$$\% \text{ Inhibition of Ulceration} = \frac{\text{Ulcer Index}_{\text{control}} - \text{Ulcer Index}_{\text{test}}}{\text{Ulcer Index}_{\text{control}}} \times 100.$$

RESULTS

Table 1
Physical Properties of Aceclofenac prodrugs

S.No	Compound	Solubility	Colour	Melting Point	I.R Spectra	N.M.R Spectra
1	Aceclofenac	Methanol	White	135-143° C	OH of COOH-3028, 2° NH-3277, 3319.	3.9δ-4.5 δ (s, 4H) 6.2-7.3δ-(m, 7H) 7.5δ-(s, 1H) 13.0 δ (s, 1H)
2	PEG 1500-Aceclofenac	Dichloro methane	Light Pink	230-241° C	C=O-1629, C-O-C-1159, CH-str-2850, C-O str- 1186, 2°NH-3327.	1.1-3.0δ-(m, 18H), 3.2-3.6δ (m, 8H), 5.5-7.6δ (s, 14H), 7.8-8.0 δ (s, 2H).
3	PEG 1500-Glycine-Aceclofenac	Dichloro methane	Red	235-245° C	2°NH-3124, 3572, C-O-C-1157, C=O 1629, CH-str-2850, C-N str-1311.	1.1-2.8δ (m, 16H) 2.9-3.6δ (s, 8H) 5.5-7.8δ (m, 14H) 7.9-8.0δ(s, 2H), 8.1-8.2 δ (s, 2H)
4	PEG 6000-Aceclofenac	Dichloro methane	Light Pink	216-225° C	C=O-1626, C-O-C-1157, CH-str-2850, C-O str-1186, 2° NH-3327.	1.1-3.0δ-(m, 18H), 3.2-3.6δ (m, 8H), 5.5-7.6δ (s, 14H), 7.8-8.0 δ (s, 2H).
5	PEG 6000-Glycine-Aceclofenac	Dichloro methane	Brick Red	196-212° C	2°NH-3124, 3630 C-O-C-1159, CH str-2850, C-O str-1186, C=O-1627, C-N str 1311.	1.1-2.8δ (m, 16H) 2.9-3.6δ (s, 8H) 5.5-7.8δ (m, 14H) 7.9-8.0δ(s, 2H), 8.1-8.2 δ (s, 2H)

Figure 1
I.R Spectra of Aceclofenac

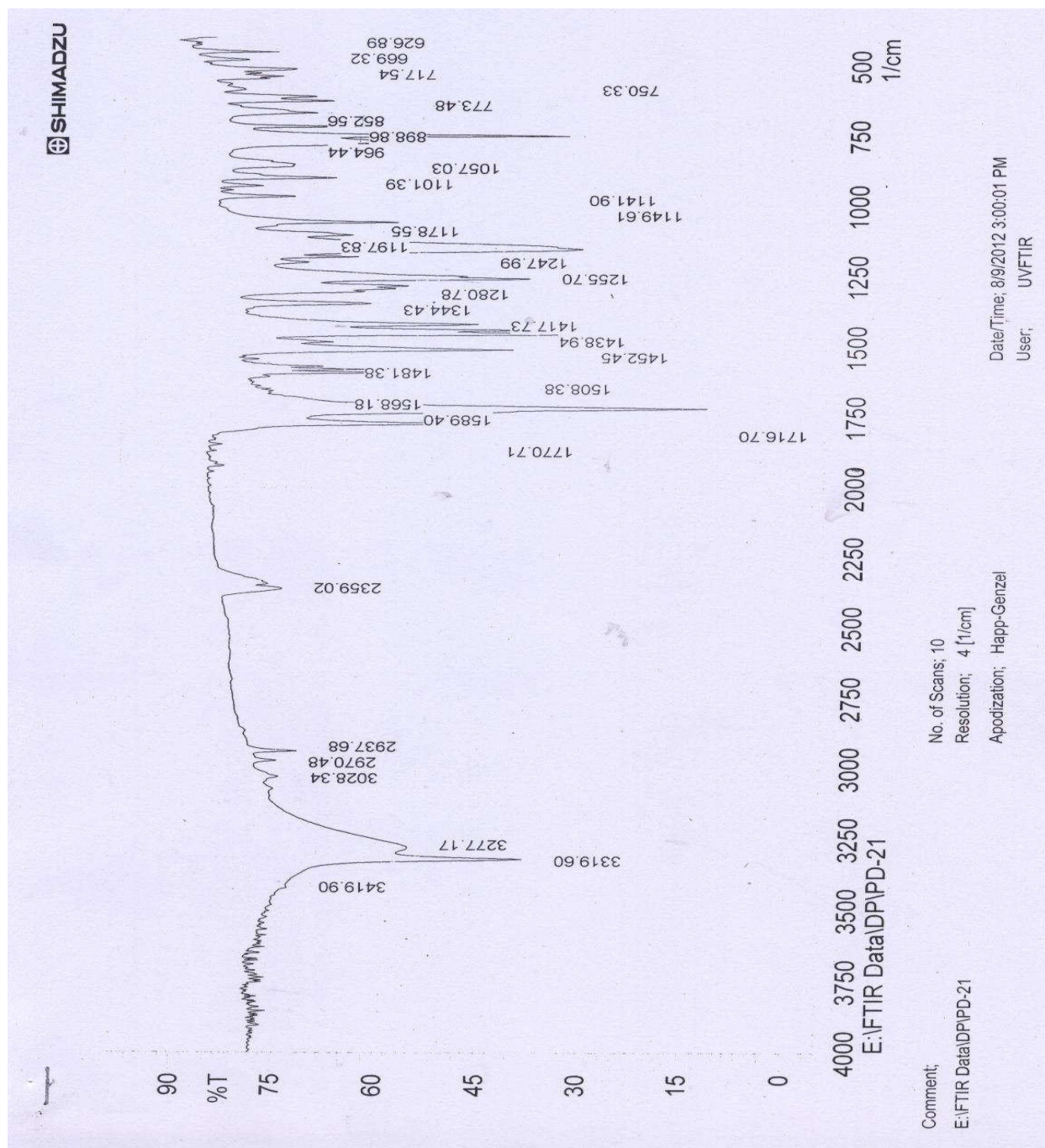
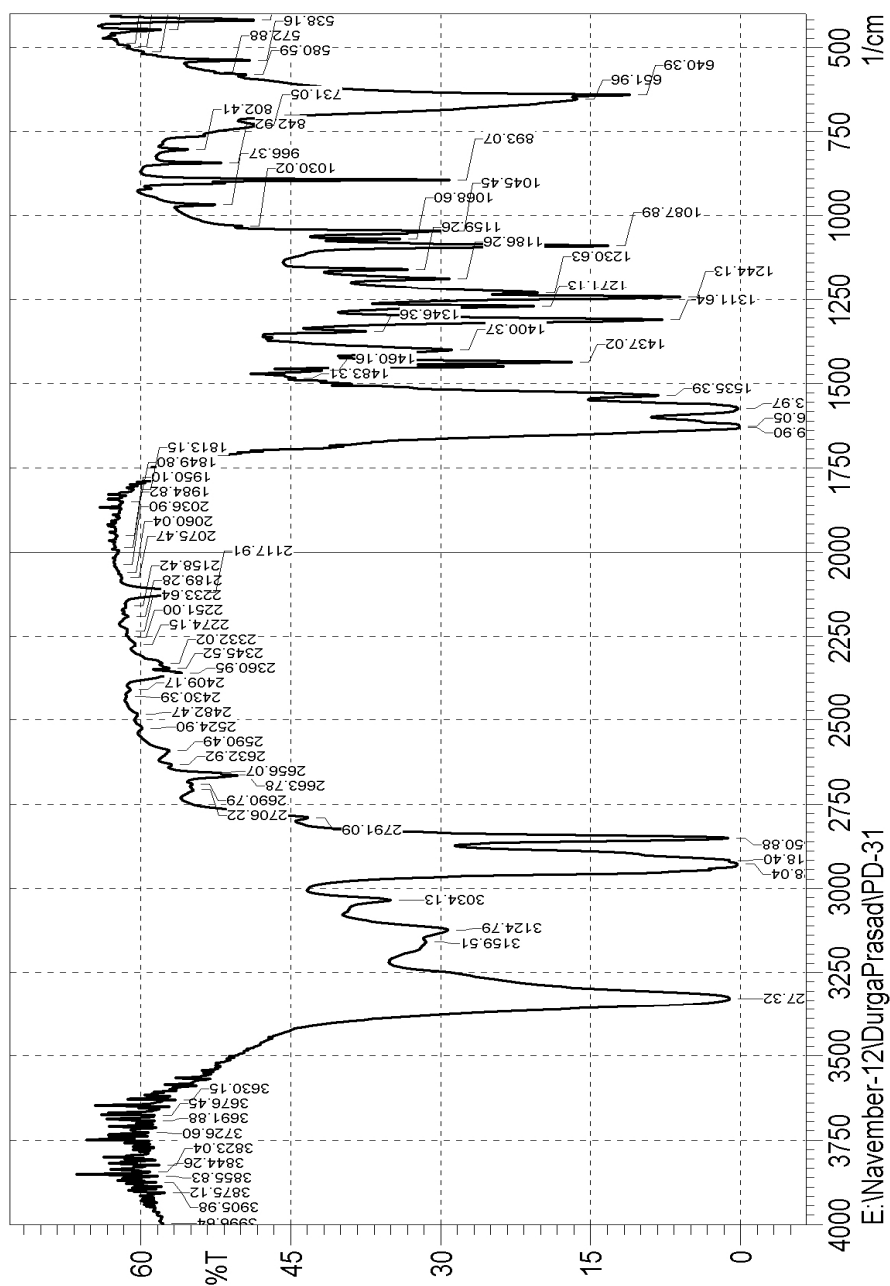


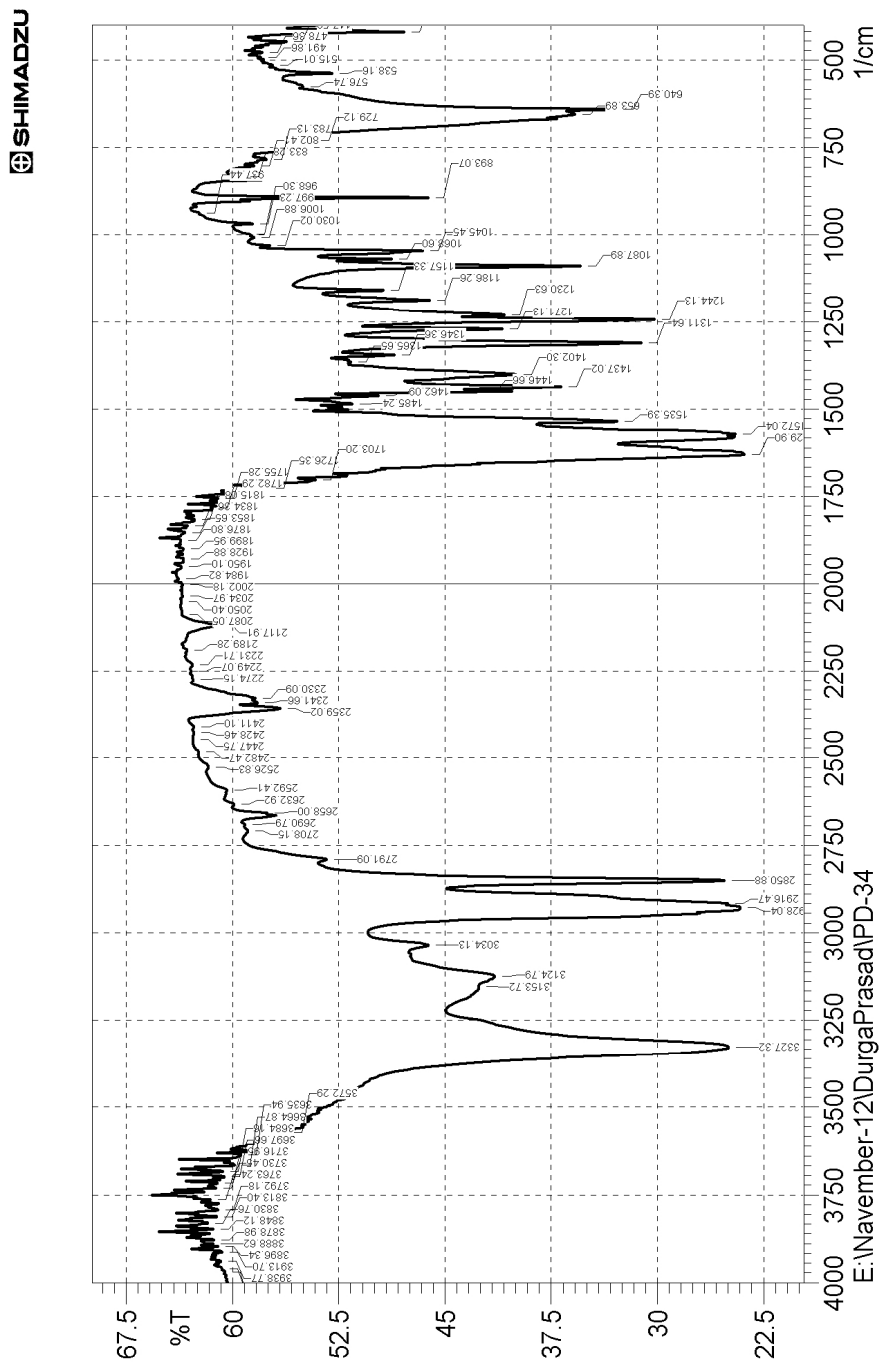
Figure 2
I.R Spectra of PEG 1500/6000-Aceclofenac

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Figure 3
I.R Spectra of PEG 1500/6000-Glycine-Aceclofenac



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Figure 4
N.M.R Spectra of Aceclofenac

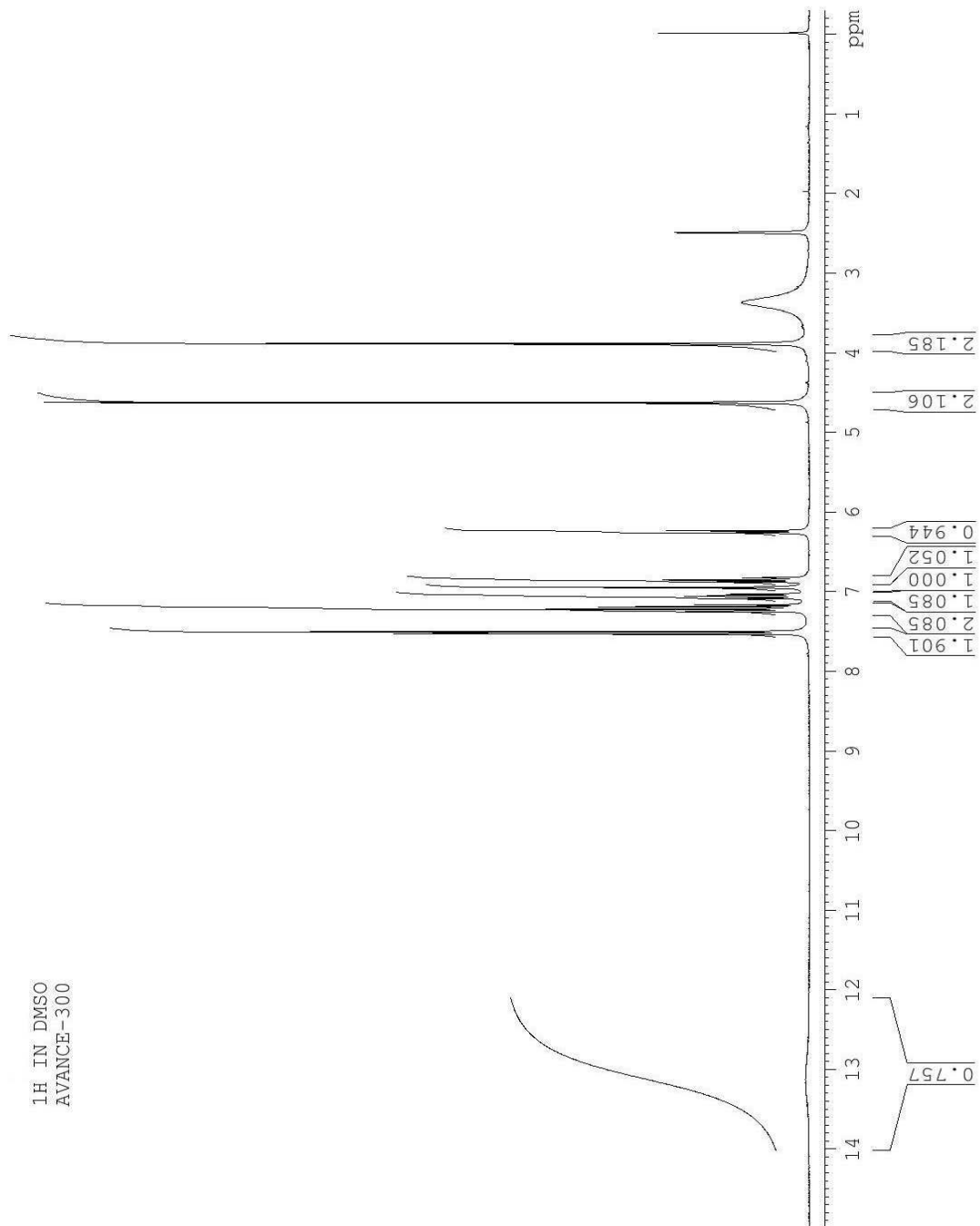


Figure 5
N.M.R spectra of PEG 1500/6000-Aceclofenac

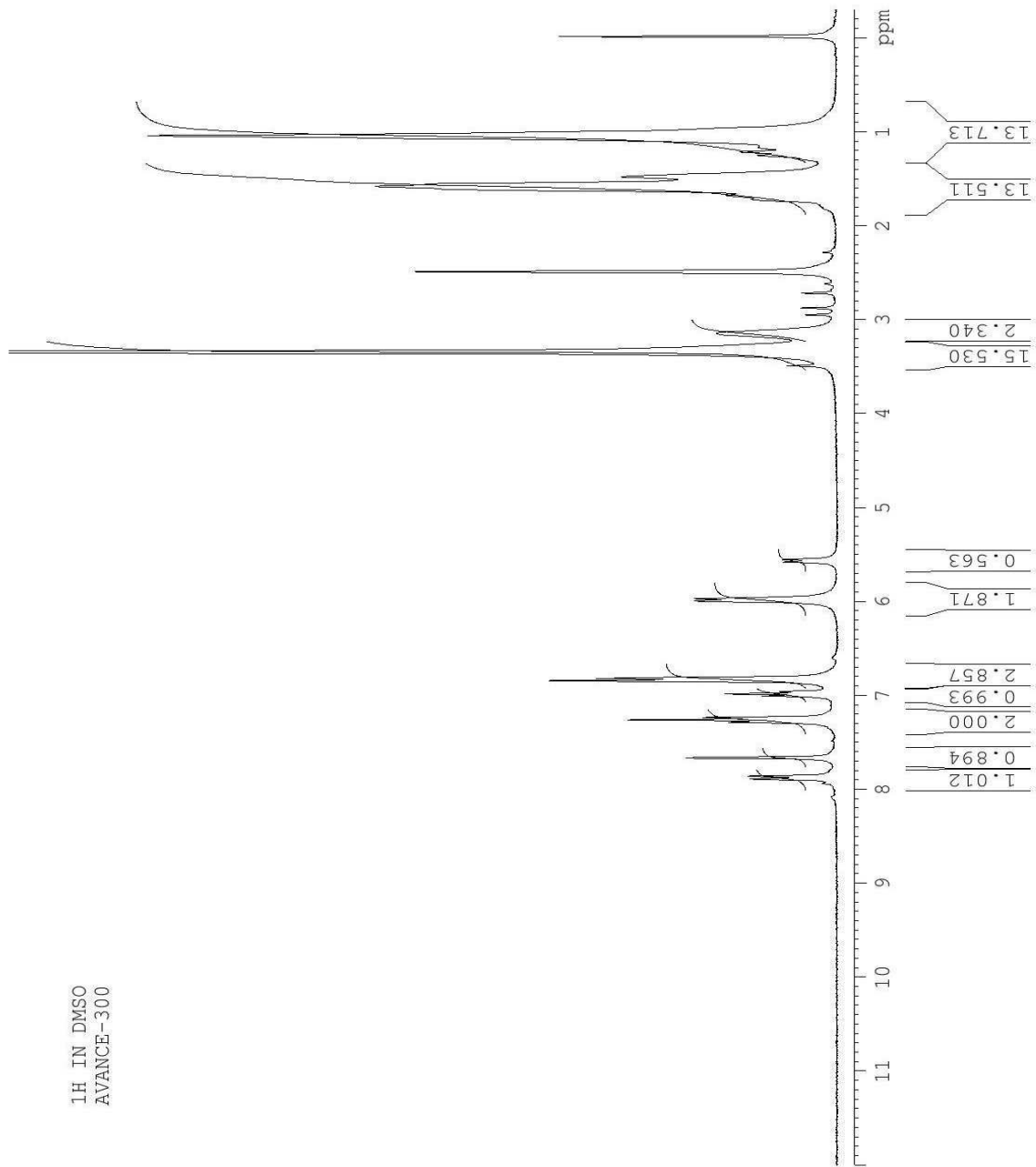
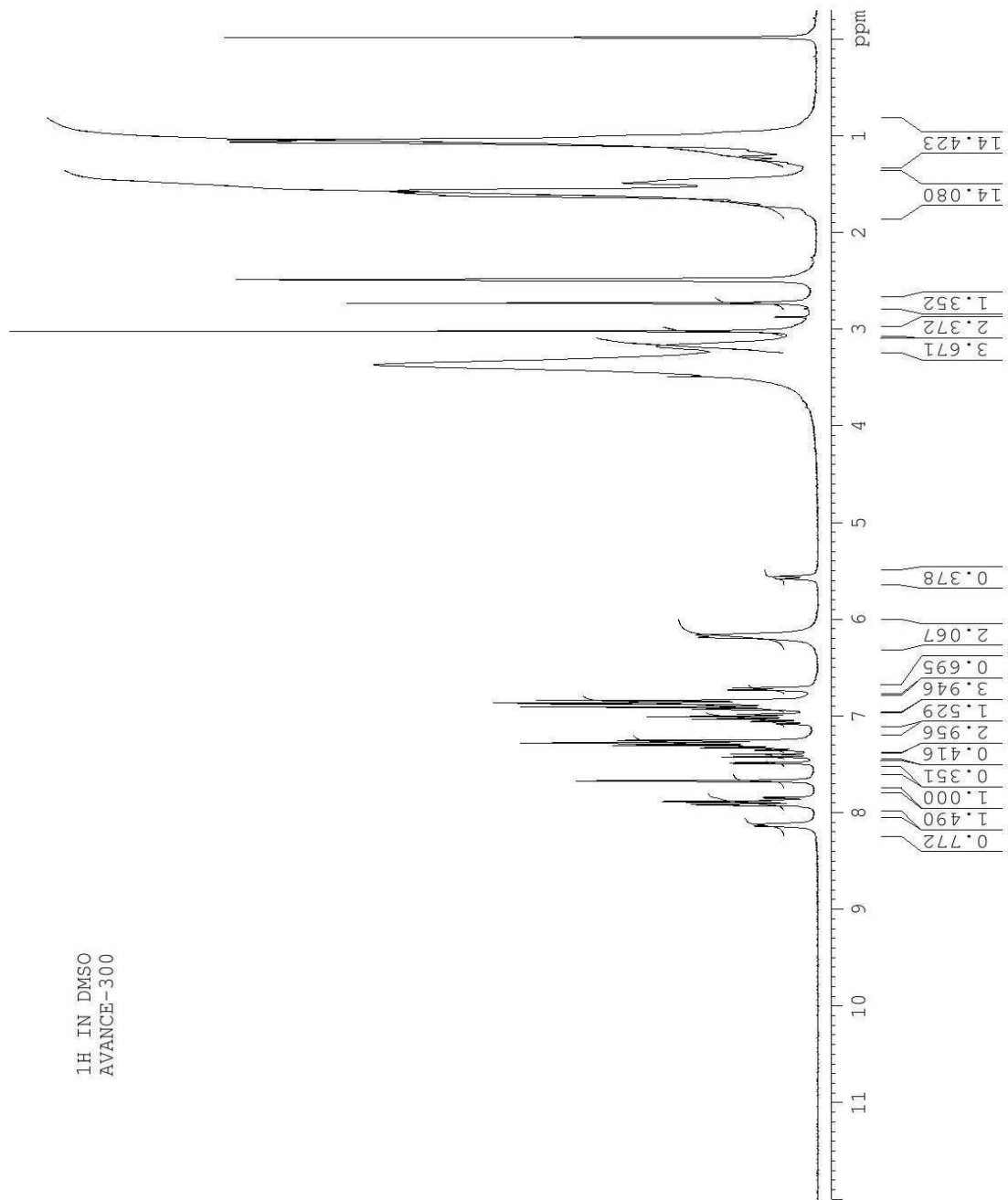
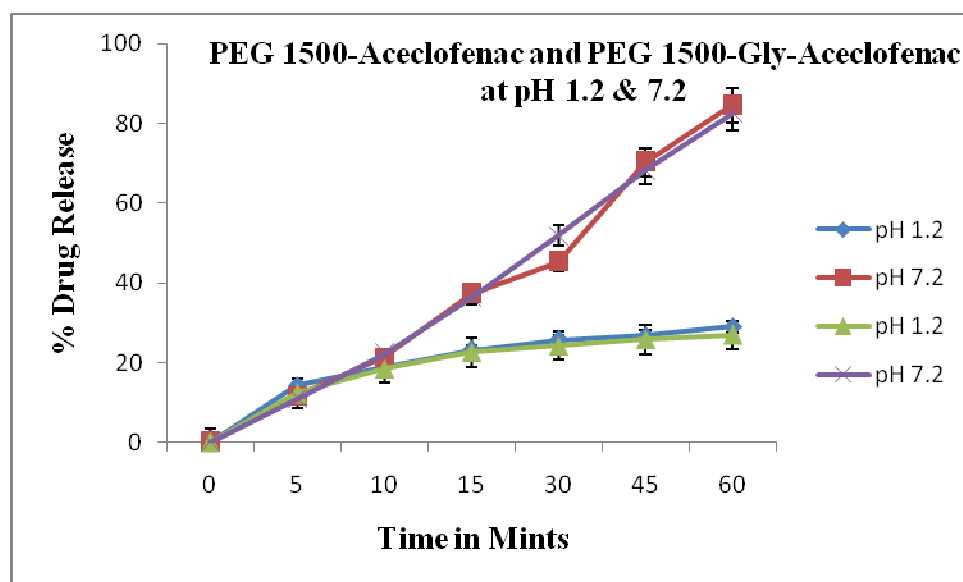


Figure 6
N.M.R spectra of PEG 1500/6000-Glycine-Aceclofenac



Graph 1

In vitro drug release profile of PEG 1500 prodrugs at pH 1.2 and 7.2.



Graph 2

In vitro drug release profile of PEG 6000 prodrugs at pH 1.2 and 7.2.

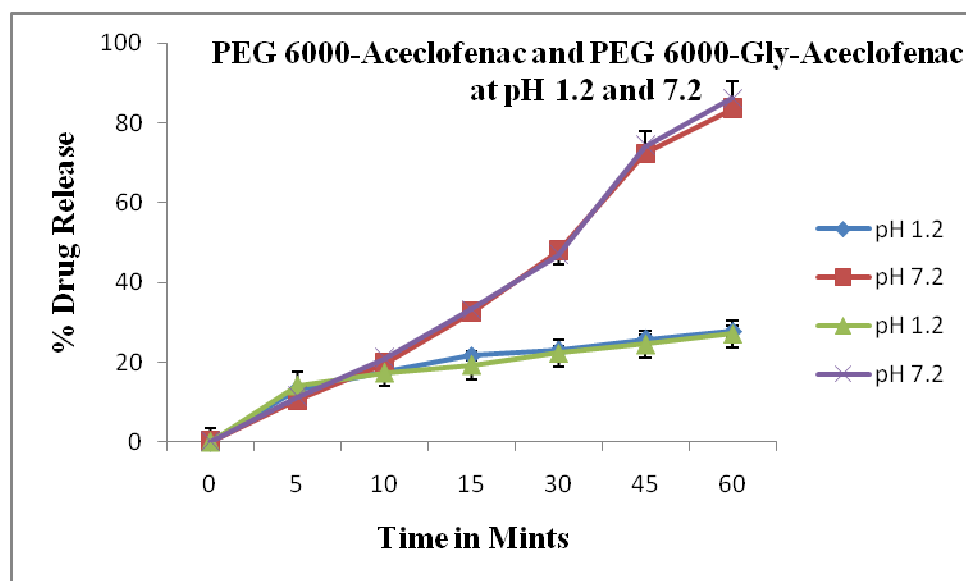


Table 2

Anti inflammatory Activity

Groups	Change in Paw volume (ml) mean±SEM & % Inhibition		
	1 hr	3 hr	6 hr
Control	0.47±0.01	0.59±0.01	0.65±0.02
Aceclofenac	0.30±0.02 (36.17)	0.42±0.01 (21.81)	0.33±0.02 (49.23)
PEG 1500-Aceclofenac	0.31±0.02 (34.04)	0.45±0.01 (23.73)	0.37±0.01 (43.08)
PEG 1500-Gly-Aceclofenac	0.39±0.01 (17.02)	0.51±0.03 (13.56)	0.36±0.02 (44.62)
PEG 6000-Aceclofenac	0.34±0.01 (27.66)	0.46±0.02 (22.03)	0.35±0.01 (46.15)
PEG 6000-Gly-Aceclofenac	0.38±0.01 (19.15)	0.53±0.01 (10.17)	0.39±0.02 (40.00)

Values are mean±SEM, n=6, one way ANOVA p<0.05 vs control.

Graph 3
Anti inflammatory activity of Aceclofenac prodrugs

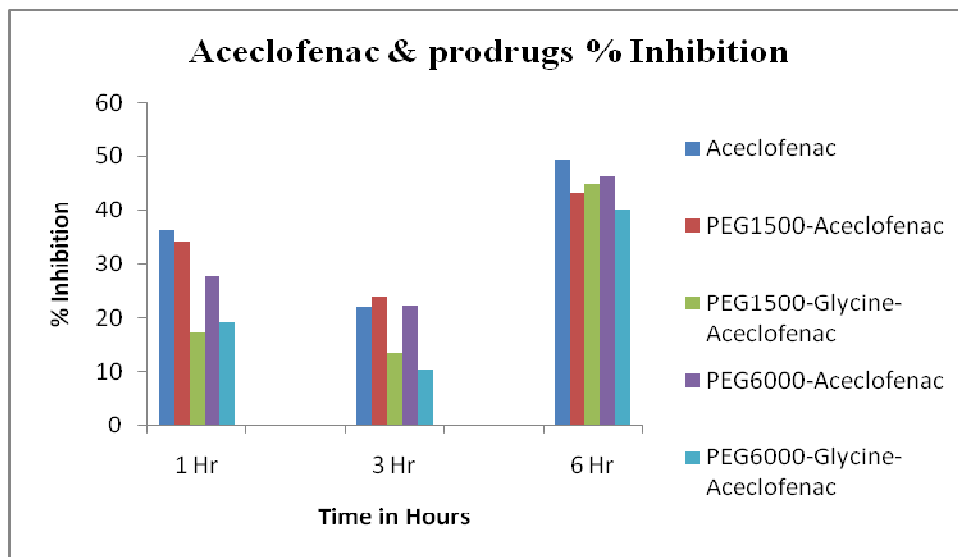
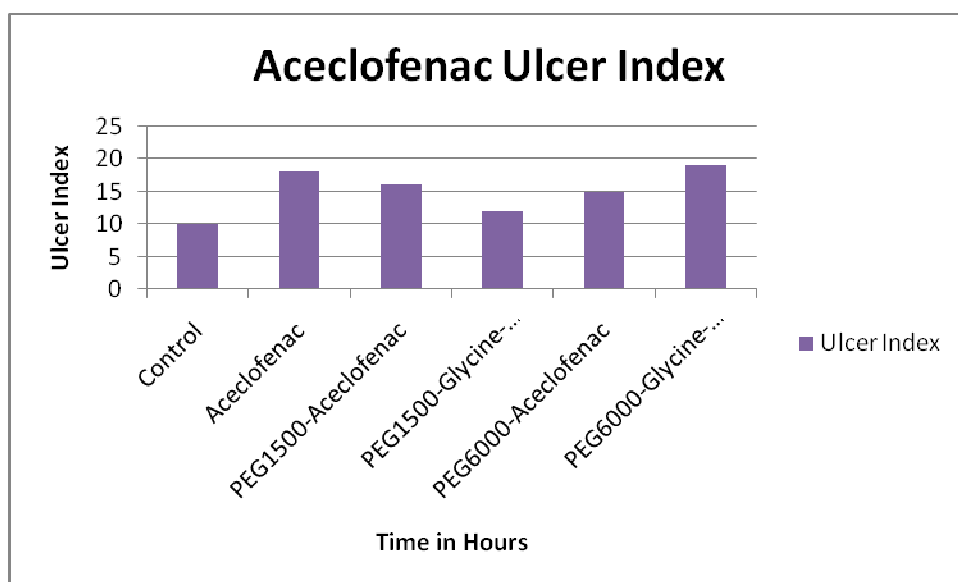


Table 3
Gross Ulcer Index of Aceclofenac prodrugs

S.No	Treatment	Ulcer Index
1	Control	10
2	Aceclofenac	18
3	PEG 1500-Aceclofenac	16
4	PEG 1500-Gly-Aceclofenac	12
5	PEG 6000-Aceclofenac	15
6	PEG 6000-Gly-Aceclofenac	19

Graph 4
Ulcer Index of Aceclofenac prodrugs



DISCUSSION

According to (S.No 2 and 4 of table 1) the spectral data obtained for prodrugs of aceclofenac, COOH group of drug had formed an ester with PEG 1500/6000 and the structures are confirmed. Spectral data (S.No 3 and 5 of table 1) confirmed that the carboxyl group of drug had formed an amide linkage with PEG 1500/6000-glycine. The *in vitro* dissolution studies result obtained for the prodrugs of PEG 1500 and PEG 1500-glycine at pH 1.2 and 7.2 revealed that the drug release was higher at pH 7.2 than at pH 1.2. The result obtained from drug release studies for the prodrugs of PEG 6000 and PEG 6000-glycine, indicated a higher amount of drug release at pH 7.2 than at pH 1.2. The result obtained from aceclofenac prodrugs with respect to anti inflammatory activity was comparable to the standard drug and are equipotent. The result obtained from ulcer index of aceclofenac prodrugs indicated that

majority of them were more protective against ulcer formation as compared to standard drug.

CONCLUSION

Aceclofenac prodrugs prepared by PEGylation have retained their anti inflammatory activity as well have shown good ulcer protecting activity, when compared with the standard aceclofenac drug. Hence, to overcome adverse effects this is one approach.

ACKNOWLEDGEMENT

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