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Synthesis and Ulcer reducing activity studies of some Peptide derivatives of Aspirin

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Abstract:

*Tetrapeptide derivatives of Aspirin (**1** to **4**) were synthesized by the following methods. Aspirin was prepared by acetylation of salicylic acid using Ac_2O / AcOH. Aspirin was coupled with aminoacid amide and dipeptide amide using its p-nitro phenyl (N_P) ester. The ester (Aspirin – ON_P) was prepared by treating p-nitro phenol and DCC in EtOAc and was precipitated by using EtOH. The synthesis of dipeptide amides were carried out in solution by stepwise elongation of the peptide chain from the C-terminal aminoacid by coupling one aminoacid at a time using DCC/HOBt method. Boc-group was used from N^{α} protection of all amino acids. The Boc-group cleavage was carried out using 50% TFA / CH_2Cl_2. The amidation of C-terminal aminoacid was carried out by treating the corresponding Boc – aminoacid – ONp esters with dry NH_3 in presence of DCC and HOBt. Their gastric ulcer inducing property was studied by histopathological method.*

***Keywords*:** Aspirin – ONp, Boc – N_3 (t-butyl azido formate), HOBt (1-Hydroxy benzotriazole), DCC (N, N^1 – Dicyclo hexyl carbodiimide) and DCU (N, N^1 – Dicyclohexyl urea).

1. Introduction

Salicylic acid was found to possess analgesic activity[1] and it was used for the treatment of integumental pain, headache and reduce fever. Nevertheless, it was not used medicinally due to its bad effect of producing gastric disturbances. But, their derivatives are found to be useful. Aspirin is used for musculo skeletal disorders. In a sufficiently large dose, aspirin acts as an anti rheumatic and antiplatelet agent. In a single dose, aspirin produce only analgesic action. Use of analgesics requires an understanding of the biochemical and physiological mechanism of analgesics[2].To improve the solubility and to reduce the side effects of Aspirin[3] various peptide derivatives of aspirin were synthesized by attaching the peptide fragments to the carboxyl group of aspirin[5, 6,7,8,9]

Aspirin prepared from Salicylic acid could be obtained in highly pure form after recrystallisation twice or thrice in hot water. The glycine amino acid sample which usually contains small quantities of glycylglycine was removed after N^{∞}-protection using Boc group by using column packed with silica gel and solvent system A. [Chloroform : Methanol : Acetic Acid (40 : 2 : 1)].

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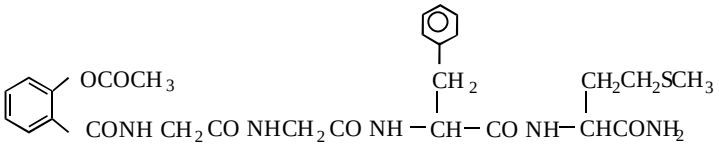
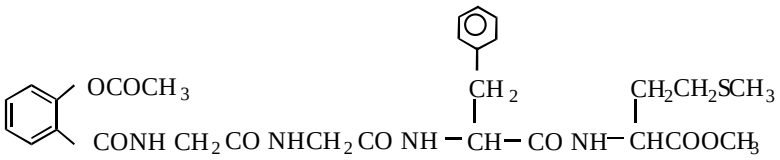
Aspirin – ONp[10] and Boc-aminoacids[11] were prepared using the reported procedure. Boc-aminoacid-NH_2 was prepared using Boc-amino acid / dry NH_3 / DCC / HOBt and was obtained in about 85% yield. The structure of Aspirin, Aspirin-ONp, Boc-Leu, Boc-Met, Boc-Leu-NH_2, Boc-Met NH_2, Boc-Phe, Boc-Phe-NH_2 were confirmed by IR data.

The tetrapeptide derivatives of aspirin ,Asp – Gly – Gly – Phe – Met – NH_2 (**1**) , Asp – Gly – Gly – Phe – Met – OMe (**2**)
Asp – Gly – Gly – Phe – Leu – NH_2 (**3**), Asp – Gly – Gly – Phe – Leu – OMe (**4**) were synthesized by attaching the peptide fragments to the carboxyl group of aspirin by DCC / HOBt[12] method and were homogenous. The structure of Asp – Phe – Leu – NH_2, Asp – Gly – Phe – Leu – NH_2, Asp – Gly – Gly – Phe – Leu – NH_2, Asp – Phe – Met – NH_2, Asp – Gly – Phe – Met NH_2, Asp – Gly – Gly – Phe – Met – NH_2 were confirmed by NMR spectral data. Their analgesic potency and their ulcer inducing property were determined.

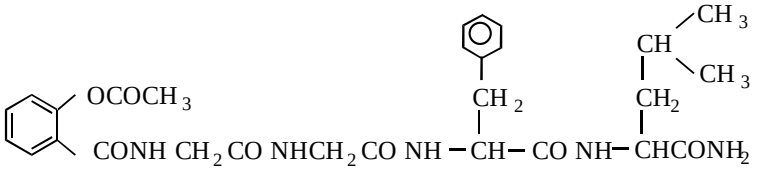
In IR spectrum, no broad band around 3345cm^{-1} shows the absence of O–H group in Boc-aminoacid. Two sharpbands at 3400cm^{-1} and 3193cm^{-1} observed may correspond to the asymmetric and symmetric NH stretching frequencies of the primary amide. The band at 1720cm^{-1} observed may be due to carbonyl group. The sharp band around 3348cm^{-1} may be due to the NH stretching of the secondary amide. Further proof from the NMR spectrum of the peaks at δ2.27, δ8.3, δ7.95 shows the presence of – $COCH_3$, - NH - , and – NH_2 groups respectively. These details confirms the following structure for the products (1, 2, 3 and 4)



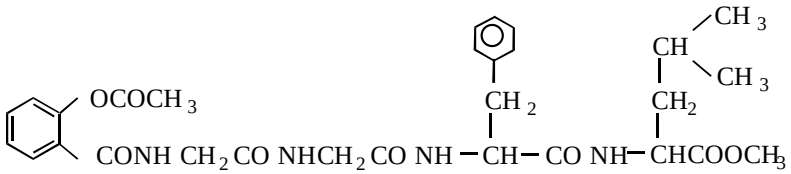
Glycyl – Glycyl – Phenylalanyl – Methionine amide of Aspirin **(1)**



Glycyl – Glycyl – Phenylalanyl – Methionine ester of Aspirin **(2)**



Glycyl – Glycyl – Phenylalanyl – Leucine amide of Aspirin **(3)**



Glycyl – Glycyl – Phenylalanyl – Leucine ester of Aspirin **(4)**

2. Experimental

2.1. **Synthesis of tetrapeptide amide of aspirin:-**

Synthesis of Asp-Gly- Gly –Phe-Met-NH_2 (1)

The tetrapeptide derivative of Aspirin , Aspirin –Glycine-Glycine-Phenylalanine -Methionine -amide (**1**) is prepared by the following methods .Boc –Met (**1a**) (2.5g,10m.moles) and HOBt (1.5g,11m.moles)in CH_2Cl_2 (40ml) and DMF (10ml), DCC (2.1g, 10.5 mmoles) in CH_2Cl_2 (20ml) were added and the mixture was stirred for thirty minutes . Then dry NH_3 was passed through the solution and stirred for 4-5 hrs till saturation. The Boc-Met NH_2 **(2a)** obtained was deprotected by treating with a 1:1 mixture of TFA/CH_2Cl_2 for 30 minutes. Then the residue was treated with 5M HCl/THF for 30 minutes to get HCl Met NH_2 .This HCl. Met NH_2 was dissolved in dry DMF (12 ml) and neutralized with Et_3N .To this was added a solution of Boc-Phe (2.1g, 8 mmoles) and HOBt (1.1g, 8 mmoles) in CH_2Cl_2 (15ml). The mixture was cooled to 0°C and to this a cold solution of DCC in CH_2Cl_2 (15ml) was added drop wise under stirring to get Boc-Phe-Met-NH_2 (**3a**).

The Boc-Phe-Met-NH_2 was deprotected by the above procedure to get HCl.Phe-Met-NH_2 . This was dissolved in dry DMF(8 ml) and neutralized with Et_3N and added a solution of Boc-Gly(1g, 5.5 mmoles) and HOBt (0.8g, 5.5 mmoles) in CH_2Cl_2 (10ml). The mixture was cooled to 0°C and to this a cold solution of DCC (1.1g, 5.5 mmoles) in CH_2Cl_2 was added dropwise under stirring to get Boc-Gly-Phe-Met-NH_2 (**4a**). Then this was deprotected and another aminoacid Glycine was introduced to get Boc-Gly-Gly-Phe-Met-NH_2 (**5a**).

The compound (**5a**) was deprotected by treating with a 1:1 mixture of TFA/CH_2Cl_2 (5ml) for 30 minutes at room temperature .The residue was treated with 5M HCl/THF for 30 minutes to get HCl.Gly-Gly-Phe-Met-NH_2 . This was dissolved in dry DMF and neutralized with Et_3N.To this was added a solution of aspirin-ONp (0.8g, 2.5 mmoles) and HOBt (0.3g, 2.5 mmoles) in CH_2Cl_2 (5ml) and stirred for 24h at room temperature to get Asp-Gly-Gly-Phe- Met-NH_2(**1**) and was obtained in 73% yield(scheme 1,table 1).

The other tetrapeptide amide Asp-Gly-Gly-Phe-Leu-NH_2 (**3**) was also prepared by the same methodology and was obtained in 76%yield (scheme I, table I).

2.2. **Synthesis of tetrapeptide ester of aspirin**

Synthesis of Asp-Gly-Gly-Phe- Met-OMe(2)

Super dry methanol(10ml) was cooled in an ice salt bath to -5^{o}C and with stirring thionyl chloride (2.6ml) was added during 20 minutes. Methionine (**6a**) (1.4 g, 10 mmoles) was then added in small portions during 20 minutes and stirred vigorously for 2h below 0°C and slowly allowed to attain room temperature to get Met–OMe.HCl (**7a**).A stirred solution of **7a** (3.6g, 18mmoles) in dry DMF (30ml) was neutralized with Et_3N. To this was added a solution of Boc–Phe (2.7 g, 10 mmoles) and HOBt (1.4 g, 10 mmoles) in CH_2Cl_2 (15 ml). The mixture was cooled to 0°C and to this a cold solution of DCC (2.1g, 10 mmoles) in CH_2Cl_2 (15 ml) was added dropwise under stirring. The reaction mixture was then stirred for about 1 h at 0°C and for about 24h at room temperature to get Boc- Phe- Met-OMe (**8a**).

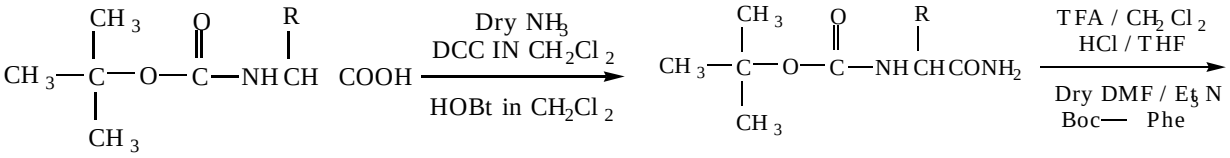
The compound (**8a**) was deprotected by the usual procedure and the HCl- Phe- Met-OMe was dissolved in dry DMF (12 ml) and neutralized with Et_3N. To this was added a solution of Boc-Gly (1.4 g, 8 mmoles) and HOBt (1.1 g, 8 mmoles) in CH_2Cl_2 (15 ml). The mixture was cooled 0°C and to this a cold solution of DCC (1.6 g, 8mmoles) in CH_2Cl_2 (15 ml) was added dropwise under stirring . The reaction mixture was then stirred about 1h at 0^{o}C and for about 15h at room temperature to get Boc- Gly-Phe- Met-OMe (**10a**). The compound (**10a**) was deprotected and the hydrochloride salt was dissolved in dry DMF (8ml) and neutralized with Et_3N. To this was added a solution of Boc-Gly (1g,5.5m.moles) and HOBt (0.8g, 5.5 mmoles) in CH_2Cl_2 (10ml).

The mixture was cooled to 0^{o}C and to this a cold solution of DCC (1.1g, 5.5 mmoles) in CH_2Cl_2 (10ml) was added dropwise under stirring. The reaction mixture was then stirred for about 1h at 0°C and for about 15h at room temperature to get Boc-Gly- Gly- Phe- Met-OMe(**12a**).The compound (**12a**) was deprotected and the HCl. Gly-Gly-Phe-Met-OMe was dissolved in dry DMF (5ml) and neutralized with Et_3N. To this was added a solution of Aspirin – ONp (1.0 g, 3 mmoles) in CH_2Cl_2 (5ml). The reaction mixture was stirred for 24h at room temperature to get Aspirin-Gly-Gly-Phe-Met-Me (**2**) and was obtained in 75% yield(scheme 2,table 1).The same procedure is adopted to get Aspirin-Gly-Gly-Phe-Leu-OMe (**4**) and was obtained in 75% yield (scheme II, table I).

Table I: Characterization data of the compounds

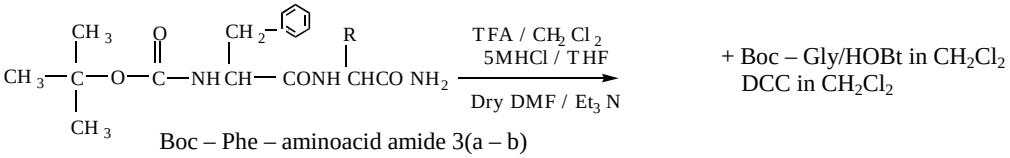
Compounds	M.P.	Yield(%)	Rf_A	Rf_B
Aspirin	135-137°C	92	----	----
1	175-178°C	73	0.56	0.51
2	116-118°C	75	0.45	0.59
3	165-168°C	76	0.45	0.52
4	137-140°C	75	0.45	0.68

Scheme 1

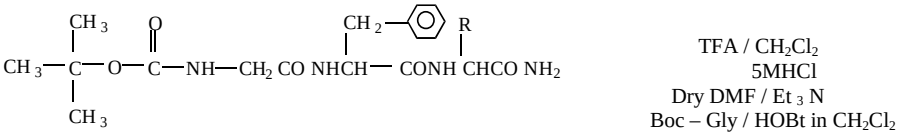


Boc – amino acid 1(a – b)

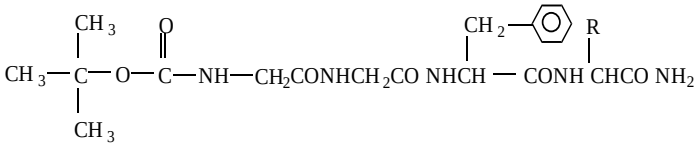
Boc – amino acid amide 2(a – b)



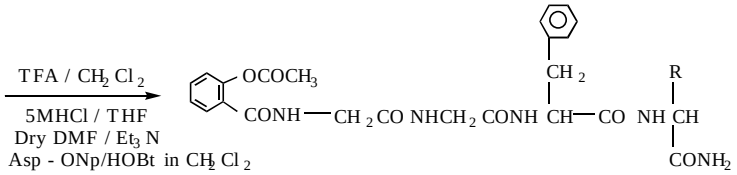
Boc – Phe – aminoacid amide 3(a – b)



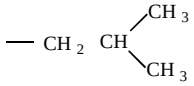
Boc – Gly – Phe – amino acid amide 4(a – b)



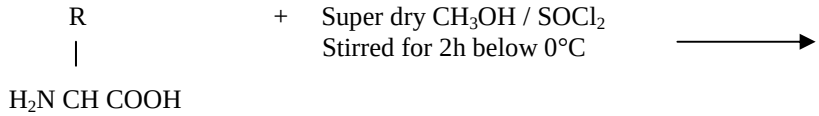
Boc – Gly – Gly – Phe – aminoacid amide 5 (a – b)



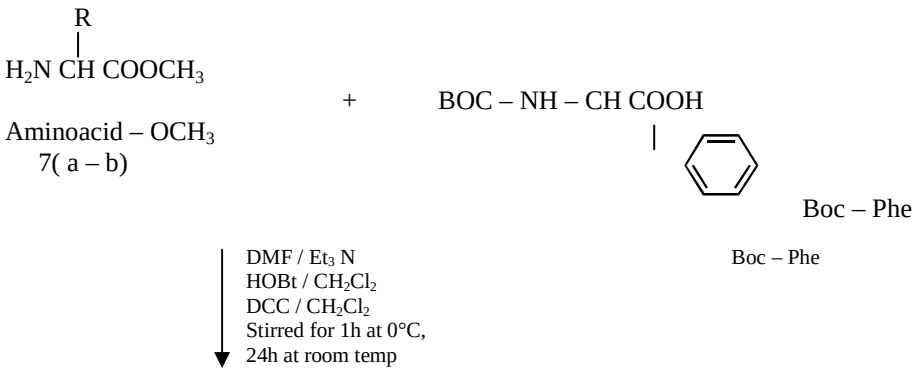
Asp – Gly – Gly – Phe – amino acid – NH_2 1 and 3

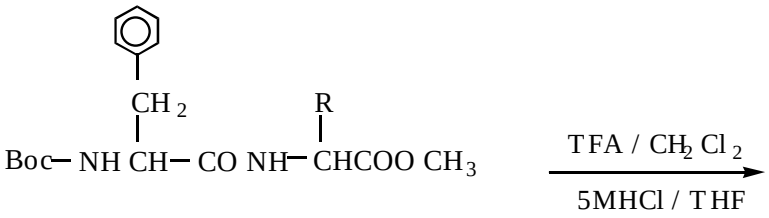
1a, 2a, 3a, 4a, 5a & 1, R = $-CH_2-CH_2-SCH_3$ 1b, 2b, 3b, 4b, 5b & 3, R = 

Scheme 2



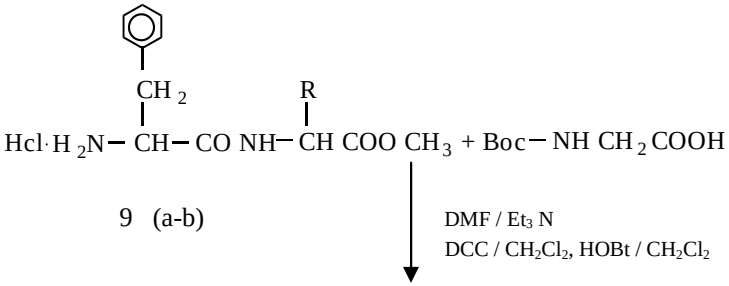
Amino acid 6(a – b)



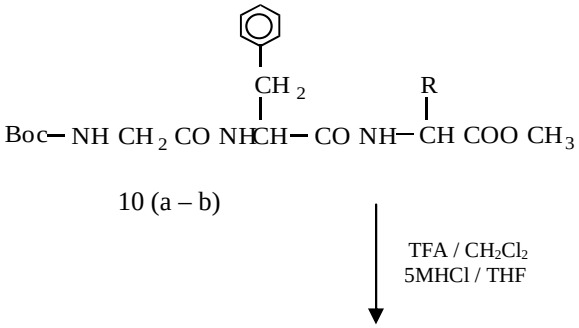


Boc – Phe – aminoacid – OCH_3

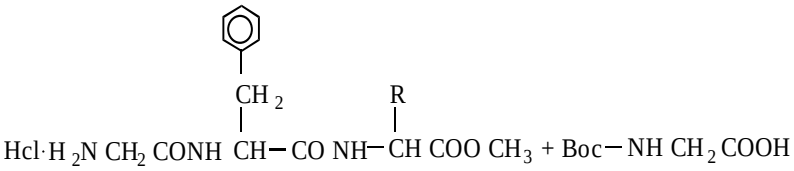
8 (a – b)



9 (a-b)

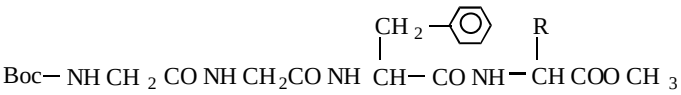


10 (a – b)



11 (a – b)

MF / Et_3N
HOBt / CH_2Cl_2
DCC / CH_2Cl_2 at 0°C



12 (a – b)

TFA / CH_2Cl_2
5MHCl / THF

13 (a – b)

DMF / Et_3N
Aspirin – ONp in CH_2Cl_2

14 (a – b)

6a,7a, 8a, 9a, 10a, 11a, 12a, 13a, &2 R = $–CH_2 – CH_2SCH_3$

6b,7b, 8b, 9b, 10b, 11b, 12b, 13b, &4 R = 

Data pertaining to a few selected compounds are as follows:

1.Asp-Gly-Gly-Phe-Met-NH_2;- M.Pt.(175-178° C);IR(KBr)3390 $cm^{-1}{}_{(A}$symm.NH),3188cm^{-1}(symm NH), 3342$cm^{-1}{}_{(GONH),1678cm}{}^{-1}$(>c=o),
^{1}HNMR ;(400MHZ) (DMSO-d_6)δ2.27(-$COCH_3$),δ7.8(-NH-) and δ8.4(-NH_2);
Elemental analysis: Glycyl – Glycyl – phenylalanyl – Methionine amide of Aspirin. $C_{27}H_{33}O_7N_5S$ (571) is found to have C 56.74%, H = 5.78% and N 12.26%.; calculated C 56.71% H5.32% and N 11.75%.

3.Asp-Gly-Gly-Phe-Leu-NH_2:- M.Pt(165 to 168° C); IR(KBr);3394$cm^{-1}{}_{(A}$symm.NH),3207$cm^{-1}{}_{(}$symm.NH), 3351$cm^{-1}{}_{(GONH),1717cm}{}^{-1}$(>c=o),
^{1}HNMR;(400MHZ)(DMSO-d_6) δ2.5(-$COCH_3$),δ8(-NH-) and δ7.95(-NH_2);
Elemental analysis: Glycyl – Glycyl – phenylalanyl – Leucine amide of Aspirin.

$C_{28}H_{35}O_7N_5$ (553) is found to have C 60.76%, H = 6.33% and N 12.66%.;
calculated C 60.2% H5.98% and N 12.32%.

1a. Boc-Aminoacid (Boc-Met) M.Pt. 109-111°C; IR (KBr); 3394cm^{-1} (asymmetric NH), 3207cm^{-1} (sym. NH), 3351 cm^{-1} (–CONH)., 1717 cm^{-1}, (>C=O),
^{1}HNMR; (400 MHz) (DMSO-d_6); δ2.5 (–$COCH_3$), δ8 (N-H) and δ7.95 (-NH_2).
Elemental analysis: $C_{10}H_{19}O_4S$ (235) is found to have C 51.06%, H = 8.09% and S 13.62%.;calculated C 50.9% H 9.12% and S 12.9%.

1b. Boc-Aminoacid (Boc-Leu)
M.P. 85-88°C; IR (KBr); 3390cm^{-1} (Asymm.NH); 3188cm^{-1} (symm.NH)., 3342cm^{-1} (CONH), 1678cm^{-1} (>C=O),
^{1}H NMR: δ2.27 (-$COCH_3$), δ8.2 (-NH-) δ8.4 (-NH_2).
Elemental analysis: $C_{11}H_{21}O_4$ (195) is found to have C 67.69%, H = 10.77% and O 32.82%.;calculated C 66.9% H10.12% and O 31.93%.

^{1}H NMR parameters of the aminoacid residues (1) and (3) are Asp – Gly (1) – Gly (2) – Phe (3) – X (4) – NH_2

Aminoacid residue	Parameters	X(4)Leu	X(4) Met
Gly (1)	δ NH	8.21	8.2
	δ CH_2	3.2	3.4
Gly (2)	δ NH	8	7.8
	δ CH_2	3.06	3.1
Phe (3)	δ NH	8	8
	δ $C^{\alpha}H$	4.5	5.3
	δ CH_2	3.6	3.4
	δ C_6H_5	7.3	7.25
– NH_2	δ	7.95	8.4
– $COCH_3$	δ	2.5	2.27

The methyl resonance of methionine at 2.04 ppm, the isopropyl resonance of leucine at 4.5 ppm[14].

3. Gastric – ulcer reducing property

Male adult Wistar Albino rats were purchased from small animal department, Trichur, Agricultural College, Kerala. The animals were adapted to the laboratory condition for a week. Water and feed was provided *ad libitum*. The animals were divided into 3 groups and treated with four peptide derivatives and it is taken for histopathological studies. In histopathological studies, an incident of gastric ulcer formation was not observed in all the four compounds. This shows that they are free from gastric ulcer inducing property.

4. Results and discussion:

*Ulcer induction evaluation***:**

Histopathological study was carried out to identify gastric ulcer inducing property. In histopathological studies, an incident of gastric ulcer formation was not observed in all the four compounds. This shows that they are free from gastric ulcer inducing property. The results of histopathological studies are given below:

Group I: Control rat

The mucosa composed of mucosal glands, laminapropria and muscularis propria appears normal. The submucosa, muscularis propria and serosa appear normal – Normal histology shown in photograph 1.

Group II: Rat treated with aspirin

There is evidence of mucosal congestion, increased numbers (proliferation) of parietal cells and focal areas of atrophy. A mucosal infiltration by easinophils is also noted. Acute non-erosive gastritis shown in photograph -2 .

Group III :(i) Rat treated with aspirin and Asp – Gly – Phe – Leu – NH_2

The mucosa, submucosa, muscularis propria and serosa appear normal. No vascular congestion (or) easinophil infiltration is noted. Complete reversal of pathological changes shown in photograph 3 .

(ii) Rats treated with aspirin and Asp – Gly – Gly – Phe – Leu – NH_2

An inflammation characterized by infiltration of easinophils into mucosa and submucosa is noted. However there is no parietal cell hyperplasia or atrophy – persistence of early acute non-erosive gastritis shown in photograph- 4.

(iii)Rats treated with the aspirin and Asp-Gly-Phe-Met-NH_2

An infiltration by eosinophils is noted in lamina propria and muscularis mucosa. Vascular congestion is also noted the submucosa, muscularis propria and serosa appear normal- Persistence of most of the pathological changes of acute non-erosive-gastritis shown in photograph -5.

(iv) Rats treated with the aspirin and Asp-Gly-Gly-Phe-Met-NH_2

The mucosa, sub mucosa, muscularis propria and serosa appear normal. No vascular congestion or easinophil infiltration is noted complete reversal of pathological changes shown in photograph- 6.

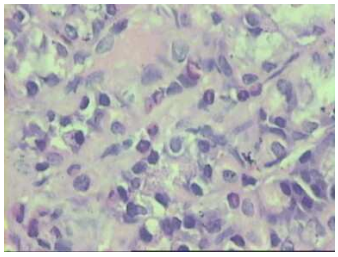


Photo-1

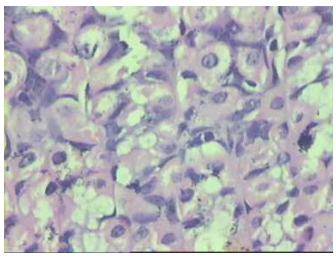


photo-2

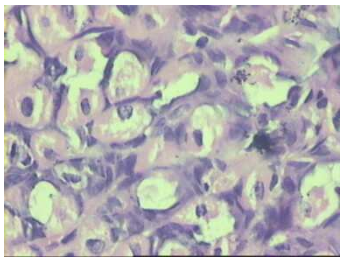


photo-3

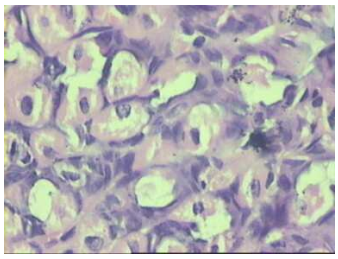


Photo-4

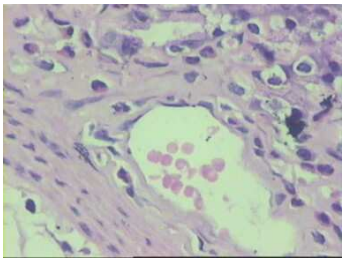


Photo-5

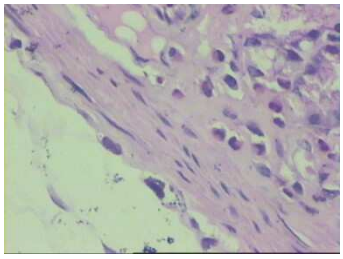


Photo-6

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