Chapter - II

STUDIES ON ACID - PEPTIC FACTOR IN EXPERIMENTAL ULCERS : ROLE OF AMLAKI (*Emblica officinalis* Linn.)

C H A P T E R - II

To study the effect of amlaki (Emblica officinalis Linn.) on the rate of gastric secretion, gastric acidity and peptic activity during experimental ulcerations, collected gastric juice samples (page, 19) were analysed by the following methodologies. Nature of ulcerogenic drugs/methods, type of animals, doses of drug and amlaki (Emblica officinalis Linn.) , route of administration, duration of anaesthesia and operative technique were essentially same as described in Chapter - I.

Materials

For acidity estimation :

a. 0.02 (N) equous solution of sodium hydroxide was prepared fresh at the time of estimation.

b. Topfer's reagent : 0.5% alcoholic solution of dimethyl amino azo benzene (Riedel-DeHae-nag-seelze-Hannover-Germany) was prepared and

stored in glass stoppered bottle.

c. 1% alcoholic solution of phenolpthalein (Merck,USA) was made and kept in a glass stoppered bottle.

For peptic activity estimation :

a. 0.01 (N) and 0.06 (N) aqueous solutions of hydrochloric acid were prepared from concentrated hydrochloric acid (Analar, BDH) with distilled water and kept in glass stoppered bottles seperately.

b. Haemoglobin solution (E.Merck) : 2% haemoglobin solution in 0.06 (N) hydrochloric acid was prepared fresh before use.

c. Trichloroacetic acid (Reanal, Budapest) : 10% solution was made by dissolving 100g trichloroacetic acid in 1 litre of distilled water and kept in glass stoppered bottle.

d. Phenol reagent (Folin & Ciocalteu's reagent) :

Sodium tungstate (Analar, BDH)	100g
Sodium molybdate (Analar, BDH)	25g
Phosphoric acid, 85-90% (BDH)	50 ml
Concentrated hydrochloric acid (Analar, BDH)	100 ml
Lithium sulphate (BDH)	150g
Bromine (E. Merck)	Few drops.

Sodium tungstate and sodium molybdate were dissolved in 700 ml of distilled water in a 2 litre round bottom flask and to this were added phosphoric and hydrochloric acids and then refluxed for 10 hours. Next lithium sulphate alongwith 50 ml of water and few drops of bromine were added. It was then boiled for 15 minutes without condenser to remove excess of bromine. The mixture was then cooled and distilled water was added to make up the volume to 100 ml and filtered. The stock solution was kept in refrigerator.

e. Alkaline reagent : 2g of sodium carbonate (Analar,BDH) was added to 100 ml of 0.1 (N) aqueous solution of sodium hydroxide (Analar,BDH).

f. Alkaline mixture : To 100 ml of alkaline reagent 1 ml of 4% aqueous potassium tartarate (E. Merck) and 1 ml of 2% aqueous copper sulphate (Analar,BDH) were added. This was prepared fresh before use.

Methodologies

Determination of volume : Volumes of gastric juice samples were individually measured with the help of a 5 ml graduated pipette and expressed in terms of ml/100g body weight of the animal/4h secretion.

Determination of acidity : 0.2ml of centrifuged blood free gastric juice sample was taken in a porcelein evaporating dish. It was diluted with 2 ml of distilled water. To it was added a drop of Topfer's reagent when a pink colour developed. It was then titrated with 0.02 (N) sodium hydroxide until a salmon pink colour (a point in between pink and yellow) was observed. To it was next added a drop of 1% alcoholic solution of phenolpthalin and the titration was continued till it turned pink.The burette reading at this point was a measure of acidity. Acidity was expressed in terms of mEq/dl and the output was calculated by multiplying each value with the respective volume of gastric juice as expressed in terms of ml/100g/4h. The aforesaid procedure to determine the gastric acidity was essentially the same as described by Verley[131].

Determination of peptic activity : Peptic activity of gastric juice was estimated employing a modified method of Anson[132]. 1 ml of 1:250 (diluted with0.01(N) hydrochloric acid) gastric juice was added to 2.5 ml of 2% haemoglobin solution in 0.06 (N) hydrochloric acid. The mixture was

incubated at 37°C for 20 minutes. Immediately thereafter an equal volume of ice cold 0.6 M trichloro acetic acid was added. The mixture tubes were then kept in ice bath for another 15 minutes and after this period the samples were centrifuged to seperate the precipiteted proteins. 1 ml of clear supernatant was used to determint the concentration of liberated amino acids following the method of Lowry et al.[133] The optical densities were measured with 'spectronic 20' absorptiometer set at 610nm against a blank similarly prepared using 0.01 (N) hydrochloric acid instead of diluted gastric juice. The peptic activity was expressed in terms of µmol tyr/ml and output was calculated by multiplying each value with the respective volume of gastric juice as expressed in terms of ml/100g/4h.

Student's 't' test was applied to evaluate the data.

Results

Results are given in and from Table 11 to Table 20.

Aspirin induced ulcer : Effect of amlaki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Table 11 shows the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in aspirin induced ulcers in albino rats. It appears from the table that no significant changes in gastric juice volume, gastric acidity and peptic activity were observed in the animals received aspirin (100 mg/kg, once in a day for consecutive three days intraperitonially) when compared to that of control animals.

In amlaki-fed group | amlaki (Emblica officinalis Linn

TABLE - 11 : Showing the effect of amlaki (*Emblica officinalis* Lin.) on rate of gastric secretion, gastric acidity and peptic activity in aspirin induced ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr. /ml	Output
CONTROL	-	25	Nil	2.69 ± 0.6	90 ±9.1	242.1 ±19.7	330 ±33.8	887.7 ±58.9
ASPIRIN	100 mg/kg	30 (5)	23.1	2.6 ±0.58	90 ±10.1	234 ±20	320 ±33.8	832 ±60
ASPIRIN + AMLAKI	100 mg/kg 1 g/kg	32 (2)	11.2	2.52 ±0.52	87 ±6	219.2 ±25	321 ±38	808.9 ±60

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

was given to the rats in the dose of 1 g/kg once in a day for consecutive three days alongwith aspirin | a low trend in the rate of gastric secretion, output in gastric acidity and peptic activity was observed when compared to that of aspirin treated group but results were not statistically significant.

Salicylic acid induced ulcer : Effect of amlaki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Results are given in Table 12.Salicylic acid, when given to albino rats in the dose of 100 mg/kg/day once in a day for consecutive three days, did not exert any significant effect in rate of gastric secretion, gastric acidity and peptic activity when compared to that of the control animals. Amlaki (Emblica officinalis Linn. treatment in the dose of 1 g/kg/day given orally once in a day for consecutive three days alongwith salicylic acid did not also bring about any significant changes in rate of gastric secretion, output of gastric acidity anf peptic activity when compared to that of salicylic acid treated group.

Paracetamol induced ulcer : Effect of amlaki (Emblica officinalis Linn. on gastric secretion, gastric acidity and peptic activity.

Table 13 shows the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in paracetamol induced peptic ulcers in albino rats. It appears from the table that paracetamol in the dose of 100 mg/kg when given to the rats intraperitonially once in a day for consecutive three days caused no significant changes in rate of gastric secretion,

TABLE - 12 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in salicylic acid induced peptic ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr./ml	Output
CONTROL	-	30	Nil	2.6 ±0.57	85 ±9	221 ±20	320 ±32	832 ±60.2
SALICYLIC ACID	100 mg/kg	32 (6)	24	2.52 ±0.58	89 ±9.1	224.2 ±21.2	330 ±30	831.6 ±59.8
SALICYLIC ACID + AMLAKI	100 mg/kg 1 g/kg	30 (3)	13.4	2.58 ±0.62	87 ±8.9	224.4 ±20.8	318 ±33.3	820.4 ±62.2

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

TABLE - 13 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in paracetamol induced peptic ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr./ ml	Output
CONTROL	-	29	Nil	2.72 ±0.7	72 ±10	195.8 ±20.2	300 ±33	816 ±72.2
PARACETAMOL	100 mg/kg	35 (6)	25	2.6 ±0.6	69.9 ±9.2	181.7 ±21.5	340 ±38	884 ±80
PARACETAMOL + AMLAKI	100 mg/kg 1 g/kg	33 (2)	8.3	2.58 ±0.61	72 ±10.1	185.7 ±20.1	322.5 ±37.2	832 ±79.5

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

gastric acidity and peptic activity when compared to that of control animals although a low trend in rate of gastric secretion, output of acidity and a high trend in the output of peptic activity were observed.

When emlaki (Emblica officinalis Linn.) was given to the rats orally (dose was 1 g/kg once in a day for consecutive three days) alongwith the aforesaid dose of paracetamol, slight changes in the rate of gastric secretion, gastric acidity and peptic activity took place but the results were not statistically significant when compared to that of paracetamol treated group.

Indomethacin induced ulcer : Effect of amlaki (Emblica officinalis Linn. on gastric secretion, gastric acidity and peptic activity.

Effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in indomethacin induced peptic ulcers in albino rats was given in Table 14. Indomethacin, when given to the rats through intraperitoneal injection (dose, 25 mg/kg once in a day for consecutive three days) did not exert any significant changes in gastric juice volume, gastric acidity and peptic activity when compared to that of control animals.Amlaki (Emblica officinalis Linn.) treatment in the dose of 1 g/kg/day given orally to the rats once in a day for consecutive three days in addition to indomethacin treatment did not also exert any significant changes in rate of gastric secretion, gastric acidity and peptic activity when compared to that of indomethacin treated group. A low trend in the rate of gastric secretion, gastric acidity was observed in this group but the results were not statistically significant.

TABLE - 14 : Showing the effect of amlaki (*Emblica officinalis* Linn.) on rate of gastric secretion, gastric acidity and peptic activity in indomethacin induced peptic ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr./ ml	Output
CONTROL	-	26	Nil	2.8 ±0.8	84 ±9.8	235.2 ±25	300 ±30.8	840 ±82.5
INDOMETHACIN	25 mg/kg	28 (2)	27.5	2.82 ±0.85	90 ±10	253.8 ±26.2	310 ±31	874.2 ±80
INDOMETHACIN + AMLAKI	25 mg/kg 1 g/kg	25 (1)	11	2.75 ±0.79	88 ±8.6	242 ±24.8	318 ±30.6	874.5 ±79.8

Results are mean ± S.E.

Brackets in perenthesis indicate the no. of animals died during experiment.

Prednisolone induced ulcer : Effect of amlaki(Emblica officinalis Linn. on gastric secretion, gastric acidity and peptic activity.

Results are given in Table 15. It reveals from the table that prednisolone, when given intraperitonially to albino rats in the dose of 30 mg/kg once in a day for consecutive three days, had no effect on the rate of gastric secretion, gastric acidity and peptic activity of ulcerated stomach. Values for gastric juice volume and gastric acidity showed a decreased trend while peptic activity value an increased trend when compared with control values but the values were statistically not significant.

Oral administration of amlaki (Emblica officinalis Linn.) to the rats in the dose of 1 g/kg once in a day for consecutive three days alongwith prednisolone in the dose as stated above did not also exert any significant change in rate of gastric secretion, gastric acidity and peptic activity when compared to that of prednisolone treated group.

Hydrocortisone induced ulcer : Effect of amlaki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Results are given in Table 16. Intraperitoneal injections of hydrocortisone (50 mg/kg/day, once in a day for three consecutive days)to albino rats did not exert any significant effect on rate of gastric secretion, gastric acidity and peptic activity when compared to that of the control animals. Amlaki (Emblica officinalis Linn.) treatment in the dose of 1 g/kg given orally to the rats once in a day for consecutive three days alongwith hydrocortisone did not also

TABLE - 15 : Showing the effect of amlaki (*Emblica officinalis* Linn.) on rate of gastric secretion, gastric acidity and peptic activity in prednisolone induced peptic ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity mEq/ml	Acidity Output	Peptic activity /umol tyr./ ml	Peptic activity Output
CONTROL	-	30	Nil	2.6 ±0.52	90 ±10	234 ±26	318 ±31.8	826.8 ±80.8
PREDNISOLONE	30 mg/kg	33 (4)	22.4	2.55 ±0.49	84 ±9.2	214.2 ±25.2	340 ±40	867 ±79.9
PREDNISOLONE + AMLAKI	30 mg/kg 1 g/kg	37 (2)	8.2	2.62 ±0.5	87 ±9.8	227.9 ±26.2	330 ±39.3	864.6 ±82.8

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

TABLE-16 : Showing the effect of amlaki (*Emblica officinalis* Linn.) on rate of gastric secretion, gastric acidity and peptic activity in hydrocortisone induced peptic ulcers in albino rats.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr./ml	Output
CONTROL	-	25	Nil	2.7 ±0.6	80 ±9.2	216 ±20.8	300 ±30.2	810 ±69.2
HYDROCORTISONE	50 mg/kg	28 (2)	26	2.52 ±0.58	89 ±10.4	224.2 ±20.9	320 ±31.9	806.4 ±75.5
HYDROCORTISONE + AMLAKI	50 mg/kg 1 g/kg	28 (0)	10	2.5 ±0.61	85 ±9.2	212.5 ±22.2	312 ±30.9	780 ±75.8

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

exert any significant change in the rate of gastric secretion, gastric acidity and peptic activity when compared to that of hydrocortisone treated group.

Phenylbutazone induced ulcer : Effect of amlaki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Table 17 shows the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in phenylbutazone induced peptic ulcers in guinea-pigs. In comparison to control data an increase trend in gastric secretion, output of gastric acidity and peptic activity was observed in phenylbutazone induced ulceration (phenylbutazone in the dose of 100 mg/kg, given to guinea-pigs orally once in a day for three consecutive days) but the increase was not statistically significant.

When amlaki (Emblica officinalis Linn.) was given to the guinea-pigs orally in the dose of 1 g/kg once in a day for consecutive three days in addition to phenylbutazone, no significant change in the level of gastric secretion, gastric acidity and peptic activity was observed when compared to that of phenyl butazone group only.

Histamine induced ulcer : Effect of amlaki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Results are given in Table 18. It appears from the table that histamine in the dose of 33 µg/mouse/day when given to the mice intraperitonially once in a day for three consecutive days produced massive ulcers in the stomach but did not exert any significant changes

TABLE-17 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in phenylbutazone induced peptic ulcers in guinea-pigs.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr. /ml	Output
CONTROL	-	30	Nil	3.6 ±0.8	92 ±10	331.2 ±30.2	357 ±35.2	1285.2 ±80.5
PHENYLBUTAZONE	100 mg/kg	32 (6)	30.2	3.75 ±0.82	90 ±9.2	337.5 ±31	362 ±35	1357.5 ±90
PHENYLBUTAZONE + AMLAKI	100 mg/kg 1 g/kg	30 (2)	12.5	3.7 ±0.81	87 ±9	321.5 ±30.8	350 ±33	1299 ±92.2

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

TABLE-18 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in histamine induced peptic ulcers in mice.

Group	Dose used.	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
					mEq/ml	Output	µmol tyr./ ml	Output
CONTROL	-	40	Nil	1.0 ±0.1	75 ±8	75 ±8.2	287 ±30	287 ±31.1
HISTAMINE	33 µg/ mouse.	46 (5)	25	0.95 ±0.09	72 ±7	68.4 ±7.4	298 ±29.2	283.1 ±30
HISTAMINE + AMLAKI	33 µg/ mouse 1 g/kg	50 (2)	9.8	0.97 ±0.08	73 ±8.2	70.8 ±8.3	290 ±30.2	281.3 ±28.9

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

in rate of gastric secretion, gastric acidity and peptic activity in comparison to control data. Amlaki (Emblica officinalis Linn.) when given to the mice orally in the dose of 1 g/kg once in a day for a consecutive three days alongwith histamine did not also exert any significant effect on volume of gastric secretion, output of gastric acidity and peptic activity when compared to that of histamine group.

Restraint ulcer : Effect of amleki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Table 19, relating to the effect of amleki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in restraint ulcers in albino rats, shows that restraint stress produced ulcers but it was not associated with any significant changes in the levels of rate of gastric secretion, gastric acidity and peptic activity in comparison to control value.These parameters again showed no significant changes when amleki (Emblica officinalis Linn.) was given to the rats orally in the dose of 1 g/kg once in a day for consecutive three days alongwith stress when compared the data obtained for restraint group only.

Shay ulcers : Effect of amleki (Emblica officinalis Linn.) on gastric secretion, gastric acidity and peptic activity.

Results are given in Table 20. It appears from the table that Shay technique for the production of gastric ulcers in albino rats did not exert any significant effect on rate of gastric secretion, gastric acidity and peptic activity when compared to the control data. Amleki (Emblica officinalis Linn.) treatment to the

TABLE-19 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in restraint ulcers in albino rats.

Group	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
				mEq/ml	Output	/umol tyr. /ml	Output
CONTROL	32	Nil	2.8 ±0.8	85 ±9	236 ±22	290 ±30	812 ±79
RESTRAINT	38 (5)	27	2.62 ±0.72	80 ±8.2	209.6 ±21.2	300 ±32.1	786 ±80.2
RESTRAINT + AMLAKI (Dose used, 1g/kg)	35 (4)	10.2	2.67 ±0.7	82 ±9	218.9 ±20	310.5 ±31.9	829 ±81.5

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

TABLE - 20 : Showing the effect of amlaki (Emblica officinalis Linn.) on rate of gastric secretion, gastric acidity and peptic activity in Shay ulcers in albino rats.

Group	No. of animals.	Ulcer index.	Volume ml/100g (4h)	Acidity		Peptic activity	
				mEq/ml	Output	µmol tyr. /ml	Output
CONTROL	30	Nil	3.0 ±0.32	75 ±8	225 ±20.2	311 ±30	933 ±90
SHAY ULCER	32 (4)	20	2.8 ±0.3	82 ±8.2	229.6 ±21.2	320.5 ±29.8	897.4 ±90.9
SHAY ULCER + AMLAKI (Dose used, 1g/kg)	33 (4)	7	2.9 ±0.32	80 ±9	232 ±23	308 ±30.2	893.2 ±87.5

Results are mean ± S.E.

Brackets in parenthesis indicate the no. of animals died during experiment.

rats in the dose of 1 g/kg orally once in a day for three consecutive days alongwith Shay stress did not also exert any significant changes in the volume of gastric secretion, gastric acidity and peptic activity when compared to that of 'Shay ulcer' group.