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Antiulcerogenic Evaluation of Aqueous Extracts of *Cichorium Intybus* and *Phyllanthus Emblica* in Normal and Aspirin-Treated Rats

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Antiulcerogenic activities of aqueous extracts of two plant drugs were studied against normal pylorous ligated and aspirin ulcerated rats. Their effect on volume of gastric juice secreted, acid output, peptic activity, mucin activity and curative ratio were recorded. The percent incidence of ulcer, average number of ulcers per stomach and ulcer index were calculated. *Cichorium intybus* showed a significant decrease in acid output and pepsin concentration in aspirin-ulcerated rats. A qualitative change in hexose and fucose concentration was found in pylorous ligated and aspirin-ulcerated rats. A 40% inhibition in ulcer formation was recorded. A significant decrease in acid output was found in animals treated with aqueous extract of *Phyllanthus emblica* in normal pylorous ligated rats but statistically non significant change in acid output was recorded in aspirin-ulcerated rats. Both normal pylorous ligated and aspirin-ulcerated rats treated with aqueous extracts of *Phyllanthus emblica* did not show any antipeptic activity. However, a significant inhibition (54%) of ulcer formation was found.

Key words: *Phyllanthus emblica*, *Cichorium intybus*, Aqueous extract, Antiulcerogenic activity.

Introduction

All the currently available synthetic antiulcer drugs fail to cure the patients completely and permanently although the rate of morbidity and mortality is greatly reduced.

The plant world is an inexhaustible source for further enrichment of the arsenal of medicinal substances with new effective preparation. It is not surprising, therefore, that some of the plants and herbs have been used to cure the peptic ulcer diseases.

Barnaulov *et al* (1984) reported that plants belonging to family Companulaceae possess antiulcer activity in experimental models. Similarly extracts of *Brassica oleracea* L. (Crucifereae) and *Solanum nigrum* L (Solanaceae) have shown antiulcer activity in aspirin-ulcerated rats (Akhtar and Munir, 1989), while aqueous and methanolic extracts, flavonoid glycosides and volatile oils extracted from *Ocimum basilicum* L (Labiateae) have shown to have significant antiulcer activity in different experimental models (Akhtar *et al* 1992). An aqueous extract of Chinese cinnamon, *Cinnamomum cassia* Blume (Lauraceae) has shown significant antiulcer activity in stress ulcers (Akira *et al* 1986).

Phyllanthus emblica fruits contain 4-9% mucic acid (Soman and Pillary 1962). The fruit is rich in vitamin C (Deb and Chandrasekhara 1960), gallic acid, $C_{16}H_{28}O_{17}$ $(COOH)_8$ and ellagic acid (Pillary *et al* 1958). *Cichorium intybus* is the most valuable source of esculetin and its glucosides (Fedorin *et al* 1974). These include esculetin, esculin cichorin, lactucin and lactacopicrin (Hee 1965). The shoots contain apigenin, luteolin 7-O-β-D-glucopyranoside, quercitrin, hyperin and epigenin 7-O-L-arabinoside (Demyanenko and Dranik 1973). The aerial parts also contain caffeic, chlorogenic, neo-chlorogenic acids, 3-feruloyl and 3-*p*-comaroyl quinic acids and dicaffeyltrataric acid (Demyanenko and Dranik 1972).

This paper deals with the effect of aqueous extracts of *Cichorium intybus* L (Compositeae) and *Phyllanthus emblica* L (Euphorbiaceae) on Shay and aspirin-induced gastric mucosal injuries in the rats.

Experimental

Plant Materials. The whole plant of *Cichorium intybus* was collected from the grain fields in Kalam (Swat) in the month of July, 1991 whilst *Phyllanthus emblica* was procured from the local market. Both the specimens were authenticated at the herbarium of PCSIR Laboratories Peshawar. The voucher specimens were preserved and catalogued in the said herbarium.

Preparation of Plant Extracts. Plant materials were first treated with *n*-hexane and then macerated in water for 48 h with occasional shaking, filtered and the filtrate was dried under vacuum. Extractive yield was calculated which was found to be 39 and 43% respectively for *Cichorium intybus* and *Phyllanthus emblica.*

Test Animals. About 50 Sprague-Dawley strain of albino rats, weighing 200±4.0 g, were used in these experiments. The animals were kept under standard conditions in the ani-

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mal house of this section and fed with standard diet. However, tap water was given *ad libitum.*

The animals were divided into four groups of 6 rats except the untreated control (n=10 and control) treated with aspirin (n=8) only. A group received aqueous extract equivalent to 0.5 g of *Cichorium intybus* kg^{-1} orally for three days twice daily alongwith aspirin treatment once daily.

Studies on normal rats. The extracts were reconstituted in water so that the yield of 1 g was present in 1 ml. The drugs were then administered equivalent to 0.5 g kg^{-1} of *Cichorium intybus* and 0.5 g kg^{-1} of *Phyllanthus emblica* three times daily for three days.

The animals were then undergone operative procedure as described herewith and the gastric juice was collected and analysed.

Aspirin-induced gastric ulceration. The modified method of Geol *et al* (1985) was used for the production of experimental gastric ulceration in rats by administering aspirin, 200 mg kg^{-1} suspended in 1% carboxymethylcellulose. The aqueous suspension of aspirin was administered with the help of a round tip cannula at 1200 h. The test drugs were administered orally at the equal dose level (0.5 g kg^{-1}) 3 h before aspirin and an equal dose 3 h after aspirin. This regimen was continued for three consecutive days and the pylorous was ligated on the fourth day.

The operative procedure adopted was that of Shay *et al* (1945) as modified by Takeuchi *et al* (1976). Feed was withheld one day prior to operative procedure.

The rats were anaesthetized with ether and the abdomen was opened through a mid line incision. The pylorous was secured and ligated with silk sutures, care being taken not to ligate the blood vessels. Abdominal walls were closed and the animals were allowed to recover from anaesthesia. After pylorous ligation drinking water was withheld and the gastric juice was allowed to collect for a period of 4 h. The rats were then killed by an overdose of chloroform and the stomach removed after clamping the oesophagus.

The gastric mucosa was washed with 3 ml of luke warm distilled water. The gastric juice and washings were homogenized and centrifuged at 5000 rpm for 5 min. The gastric juice was measured and the millilitre per 100 g body weight of the rat was calculated. The stomach was inflatted with 10 ml of 1% formaline for 10 min to fix the inner walls of the stomach. The stomach was then cut open along the greater curvature and examined for lesions in the glandular portion under 10x dissecting microscope. The maximum length of each lesion was determined and the sum of length of all the lesions in eah stomach was expressed as ulcer index as recommended by Okabe *et al* (1978) and West (1982). Number of ulcers per stomach was also recorded. Percent of ulcer incidence of each group as compared with control was calculated and curative ratio (CR) was calculated according to the formula

$$CR = \frac{\text{Control Ulcer Index - Treated Ulcer Index}}{\text{Control Ulcer Index}} \times 100$$

Determination of total acid output. Total acid output was determined by titrating 1 ml of the gastric juice in 10 ml of distilled water with 0.01N NaOH using phenolphthalein as an indicator to light pink end point. Data is expressed as microequivalents per ml of the gastric juice. Samples contaminated with blood were not included in the data (Oser 1965).

Determination of peptic activity. Peptic activity was determined by the methods of Riggs and Stadie (1943). A slight modification was made in the method that 50 ml of distilled water was added to each gram of egg white instead of 10 ml and mercuric chloride was used rather than merthiolate, remaining procedure was the same. Results expressed as mg ml^{-1} of the gastric juice and output of pepsin as mg 4 h^{-1}.

Determination of mucin activity. The ratio of total carbohydrates (hexose, hexosamine, fucose and sialic acid) to total protein was taken as the index of mucin activity. Fucose content of carbohydrates was estimated according to the method of Dische and Shettles (1948). A slight modification of Ayala *et al* (1951) was employed for the estimation of sialic acid content.

A slight modification of the method of Lusting and Langer (1931) was empolyed for the estimation of protein bound hexose. Method of Elson and Morgan (1933) was used for the estimation of hexosamine.

Total protein was determined by the use of Biuret reagent (Mehl 1945).

Statistical analysis. The data was expressed as Means±S.E.M. and Dunnett's t test was used to check its significance.

Results and Discussion

The aqueous extract of *Cichorium intybus* showed a significant decrease in acid output in aspirin treated rats. However, it did not show any inhibitory activity in normal pylorous ligated rats. A significant antipeptic activity was also found in aspirin treated rats (Table 1). A qualitative change in individual carbohydrates (hexose and fucose) and total protein was found both in aspirin ulcerated rats and pyloric ligated treated with aqueous extract of *Cichorium intybus* (Table 2).

The incidence and severity of ulcer was also reduced (Table 3).

The aqueous extract of *Phyllanthus emblica* showed significant decrease in acid output in normal pylorous ligated rats. However, pepsin concentration was not changed. In aspirin ulcerated rats, change in acid output was statistically non significant, whereas after 4 h of the treatment, a significant decrease in the peptic activity with respect to aspirin treated control was observed. (Table 1). No change in total carbohydrates or mucin activity was found with aqueous extracts of

Table 1

Effect of test extracts on volume, total acid and peptic activity of gastric juice in untreated control and aspirin treated groups of albino rats

	Treatment	Volume	Total acid out put		Peptic activity	
		m1 100 gm^{-1}	Eq $m1^{-1}$	Eq $4h^{-1}$	mg $m1^{-1}$	mg $4h^{-1}$
1.	Control 1 (Untreated) (n=10)	2.4±0.20	33±0.73	70±11	3.0±0.5	7.0±0.9
2.	Control 2 (Aspirin-treated) (n=8)	6.0±0.46	60±6.00	245±45	9.0±1.0	57±9.0
3.	*Cichorium intybus* i) A E (n=6)	2.5±0.40	38±0.63	87±57	6.8±1.2	16±3.0
	ii) A E + Aspirin (n=6)	4.6±0.40*	50±5.00*	257±40	4.0±0.5**	18±2.0**
4.	*Phyllanthus emblica* i) A E (n=6)	1.98±0.44	18±0.05++	36±10++	3.4±1.0	80±2.0
	ii) A E + Aspirin (n=6)	4.9±0.46	47±6.00	244±57	6.8±2.0	28±2.0*

* Statistically significant (P<0.10) compared to Control 2; ** = Statistically significant (P<0.001) compared to Control 2; ++ = Statistically significant (P<0.001) compared to Control 1; A E = Aqueous extract; n= Number of animals.

Table 2

Effect of test extracts on mucin activity of gastric secretion in aspirin treated albino rats

	Treatment	Carbohydrates (g ml^{-1})				Total Carbohydrates (g ml^{-1})	Total Protein (g ml^{-1})	Carbohydrates /protein ratio
		Total Hexose	Hexoseamine	Fucose	Sialic acid			
1.	Control 1 (untreated) (n=10)	1312±104	519±77	180±97	159±20	2236±193	2485±188	0.90±0.06
2.	Control 2 (Aspirin treated) 0.2 g kg^{-1} O D (n=8)	813±69	438±52	29±5	164±15	1443±193	2000±477	0.75±0.09
3.	*Cichorium intybus*							
	i) A E (n=6)	2090±983++	266±67	500±94++	251±83	3056±720++	5400±1200	0.56±0.09
	ii) A E + Aspirin (n=6)	1315±95**	211±10	144±30**	111±8	1672±111	2917±185	0.66±0.05
4.	*Phyllanthus emblica*							
	i) A E (n=6)	1913±381+	200±3	420±13++	152±8	2450±886	4200±800++	0.58±0.11
	ii) A E + Aspirin (n=6)	1377±75**	243±75	117±33**	127±21	1843±106	4284±645**	0.46±0.04

Results are means ±S.E.M.; ** = Statistically significant (P<0.001) compared with Control 2; ++ = Statistically significant (P<0.001)compared with Control 1; + = Statistically significant (P<0.01) compared with Control 1; A E = Aqueous extract, n = Number of animals.

Table 3

Effect of test extracts on gastric ulcers in rats

Test Substance	Ulcer Present	Ulcer Absent	% of ulcer incidence	Average number of ulcers per stomach	Ulcer index mm±S.E.M.	% Inhibition (C R)
1. Control Aspirin, (n=10)	9	1	90	3.0±1.00	10. 0±0.31	-
2. *Cichorium intybus* A E + Aspirin, (n=6)	5	1	83	2.0±0.49*	6.0±2.50**	40
3. *Phyllanthus emblica* A E + Aspirin, (n=6)	2	4	33	1.6±0.40*	4.6±0.40**	54

* = Statistically significant (P<0.10); ** = Statistically significant (P<0.01).

Phyllanthus emblica treatment. A significant decrease in ulcer index was found; average number of ulcers was reduced. A significant (54%) ulcer inhibition was noted (Table 3).

The present studies have demonstrated that the aqueous extract of *Cichorium intybus* and *Phyllanthus emblica* have shown antiulcer activity in aspirin ulcerated rats.

The aqueous extract of *Phyllanthus emblica* has shown strong inhibition in gastric acid secretion in Shay rats but inhibition is statistically non significant in aspirin ulcerated rats. Okabe *et al* (1974) have reported that the formation of gastric ulcers in rats in the model of Shay *et al* (1945) is dependent on the degree of acidity and not necessarily on the amount of gastric juice. This conclusion could support the back diffusion theory of H^+ ions of Takeuchi and Okabe (1983). However, Chapman (1978) states that there appears to be little correlation between severity of ulcer disease and level of acid secretion in man. Baron (1973) claims that about 50% of the patients with peptic ulcer disease fall into the normal range which limits the necessary studies as diagnostic test. Although the aqueous extract of *Phyllanthus emblica* has not decreased the secretions of acids and pepsin yet it has shown 54% inhibition of ulcer formation. The extract has also not shown any increase in mucin activity. Yet carbohydrates content of the mucin is quantitatively increased. The protective role of the mucin has been suggested by various workers (Babkin *et al* 1941; Wolf and Wolff 1943; Sanchez Palomera 1951, Zaidi and Mukerji 1958).

The ulcerogenesis by aspirin causes an increase in the level of serotonin. The drug may have exerted antiserotonin effect which at present is difficult to explain.

The aqueous extract of *Cichorium intybus* has shown decrease in gastric secretions and increase in hexose and fucose contents of carbohydrates. Thus in a two way approach for ulcer healing, the drug has shown a 40% decrease in ulcer formation. Similar findings have already been reported by Barbara *et al* (1974) for Gefarnate.

In conclusion, further investigations in different experimental models will establish the claim and if some active ingredient extractable in water is isolated, it will be a real potential in present antiulcer armamentarium.

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