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ANTIULCER EFFECT OF *ARTEMISIA ABSINTHIUM* L. IN RATS

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The extracts of *Artemisia absinthium* induced a significant decrease in volume of gastric juice, acid output and peptic activity but no effect was determined on mucin activity in acetylsalicylic acid (ASA) ulcerated rats. Moreover, they decreased the ulcer index significantly. Phytochemical analysis indicated the presence of saponins and glycosidic sugars in the extract.

Key words**:** *Artemisia absinthium*, Antiulcer activity, Saponins.

Introduction

Plants and plant products have served mankind as medicines since ancient times. The use of herbal medicines and herbal products constitute a large portion of consumers choice and continue to rise in popularity all over the world (Sanyal *et al* 1964, 1965, 1971, 1982; Elliot and Heward 1976; Al Habbal *et al* 1984; Blum 1985; Geol *et al* 1985a, b & c, 1986; Lorincz 1994; Shalita 1995; Priest 1995; Rowe 1998). *Artemisia absinthium* L., a member of the Asteraceae family, has long been used in traditional system of medicine in Pakistan and it is reported to possess well-marked antipyretic and tonic properties (Ikram *et al* 1987). Its oil is produced commercially and used as a tonic. It has stimulating effect on the digestive organs (Manjunath 1948). Keeping in view, the folkloric use of plant species in the indigenous system of herbal medicines, different semi pure extract were tested for their antiulcer effects on acetylsalicylic acid (ASA) induced ulcers in rats. In addition the effects on volume of gastric juice, acid output, peptic activity and mucin activity were also studied to evaluate the action of these extracts.

Materials and Methods

Artemisia absinthium L. was collected in July, identified and authenticated from the herbarium specimens of Peshawar Laboratories. Voucher specimens were preserved and catalogued in the said herbarium. The plant material was shade dried, powdered and stored carefully. All the solvents and chemicals used were of analytical grade. Acetylsalicylic acid was purchased from the local market. For TLC, precoated silica gel, G 60, F 254 plates (0.2mm thick, Merck) were used.

Animals. Sprague-Dawley albino rats of either sex, weighing 200 ± 4g and housed under standard conditions, were used.

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Preparation of extracts and fractionation. The air dried powdered plant material (2.0 kg) was cold percolated with 95% ethanol (three times). The combined alcoholic extracts were concentrated under reduced pressure. The crude extract thus obtained was defatted with hexane. The defatted material was then extracted successively with chloroform and carbon tetrachloride. The fraction finally obtained (in 3.75% yield) was dissolved in methanol and then passed through a charcoal - celite column to remove the coloring matter. Thin layer chromatography (TLC) of the mixture was carried out in the solvent systems ethylacetate and formic acid (1.0:0.1), chloroform, methanol and water mixture in the ratios of (65:35:10; 70:30:10 and 70:30:5) which showed the presence of six components. For visualizing the spots, the detection reagents used were $SbC1_3$ in $CHC1_3$ (15%) and anisaldehyde (0.5 ml) +EtOH (9 ml)+ H_2SO_4 (0.5 ml), followed by heating at 60 -70°C for 5 min. The crude saponin mixture was separated into five purified / semipurified fractions by chromatography on silica gel, eluting with chloroform and chloroform - methanol mixtures. Solvents were removed *in vacuo* and each of the eluent was examined for its antistress activity.

Phytochemical screening. Tests for the presence of alkaloids, glycosidic sugars, free sugars, anthraquinones and saponins in each eluent were carried out by reported standard methods (Siddiqui and Ali 1997).

ASA - induced gastric ulceration. This assay was conducted in accordance with the modified method of Geol *et al* (1985). The animals were divided into three test groups, each group contained six animals, while the untreated control group contained 10 animals. The control (ulcerated) group was given oral dose of aqueous suspension of ASA (200 mg / kg) in 1% carboxymethylcellulose (CMC). Aqueous extracts of *Artemisia absinthium* were administered orally at the dose of 5 mg / kg 3 h prior and 3 h after ASA treatment for three days. The

Table 1

Effect of different fractions (Fraction 1-5) of *Artemisia absinthium* L. on volume gastric juice secreted, acid output and peptic activity of gastric juice in untreated control and stressulcerous rats (20 h cold stressing)

S.No.	Treatment	Number	Volume of gastric juice (ml)	Acid output (mmol / h)	Peptic activity velocity constant / ml of the gastric juice / min
1.	Control Propylene Glycol 5 mg / kg i / p	10	21.00 ± 3.50	0.90 ± 0.03	0.080 ± 0.002
2.	Fraction 1 5 mg / kg in PG i / p	6	15.00* ± 3.00	0.55** ± 0.16	0.076 ± 0.004
3.	Fraction 2 5 mg / kg in PG i / p	6	13.00* ± 2.00	0.60* ± 2.00	0.040** ± 0.004
4.	Fraction 3 5 mg / kg in PG i / p	6	14.00* ± 3.00	0.70 ± 0.01	0.060 ± 0.004
5.	Fraction 4 5 mg / kg in PG i / p	6	17.50 ± 0.78	0.89 ± 0.02	0.094 ± 0.007
6.	Fraction 5 5 mg / kg in PG i / p	6	15.80 ± 1.42	0.90 ± 0.02	0.120 ± 0.030

Levels of significance compared to control *P< 0.05; **P< 0.005; PG = Propylene Glycol; i/p, intraperitoneally.

effects on healthy rats were also evaluated with 1% CMC (10 ml / kg) only. The animals were operated on the fourth day in accordance with the method of Shay *et al* (1945). The feed was withheld 18 h prior to surgery. The pylorus was ligated and gastric juice was collected for a period of 4 h. The animals were then killed and stomach was removed by clam-ping the oesophagus. The gastric juice was collected and centrifuged (5000 rpm, 5 min). The supernatant liquid was col-lected in a graduated cylinder and volume calculated (ml / 100 g b.w.). The stomachs were then inflated with 1.00% formalin (10 ml; 10 min). The average number of ulcers per stomach were recorded and percent inhibition of ulcer formation cal-culated (Okaba *et al* 1978).

Acid output. Acid output was determined by titration of volume gastric juice secreted with 0.01 N NaOH using phenolphthalein as an indicator (Oser 1965).

Peptic activity. Peptic activity was measured by the modified method of Rigges and Stadie (1933) with some modifications, as 50 ml of distilled water was used instead of 10 ml. Mercuric chloride was used as a preservative in place of thiomersalate.

Mucin activity. The ratio of total carbohydrates to total protein was calculated as an index of mucin activity. Fucose was determined by the method of Dische and Shettles (1948). Protein bound hexose was quantified by slight modification of Lusting and Langer procedure (1931). Hexosamine was estimated in accordance with the procedure of Elson and Morgan (1933). Gastric juice was heated with hydrochloric acid in boiling water bath (3 N; 16 h). Sialic acid was measured by the method of Ayala *et al* (1951). Whereas, total protein was determined in accordance with the procedure of Winzler *et al* (1948).

Statistical analysis. Significance of the values obtained was evaluated by Student's t-test.

Results and Discussion

The phytochemical studies were undertaken to evaluate the antiulcerogenic effects of purified / semipurified extracts of *A. absinthium* L. Phytochemical analysis showed the absence of alkaloids and anthraquinones but indicated the presence of glycosidic sugars and saponins, which showed the characteristic saponic properties in aqueous alcoholic extracts such as foaming, toxicity towards fish and hemolytic activity. The Liebermann-Burchard test was also used as a color test which is adopted in the Japanese Pharmacopea as identification method for crude drugs containing saponins e.g. Platycodi Radix, *Anemarrhenae rhizoma*. (Sinsaku *et al* 1981). The ASA induced ulcerated group treated with different fractions of *Artemisia absinthium* L. has shown a significant decrease in the volumes of gastric juice, decrease in acid output and peptic activity (Table 1). Peptic activity was measured according

Table 2

Effect of different fractions (Fraction 1-5) of *Artemisia absinthium* L. on percent of ulcer incidence, Average No. of ulcer per stomach, ulcer index and percent inhibition of ulcer formation in stress ulcerated rats (20 h cold stressing at 23°C)

S.No.	Treatment	Number	Ulcer present	Absent	Ulcer incidence (%)	Average No. of ulcer per stomach	Ulcer index	Inhibition (%)
1.	Control Propylene Glycol 5 mg / kg i / p	10	10	0	100	7.00 ± 0.05	18.00 ± 6.00	-
2.	Fraction 1 5 mg / kg in PG i / p	6	5	1	83	3.00** ± 1.00	8.00* ± 4.00	65
3.	Fraction 2 5 mg / kg in PG i / p	6	5	1	83	5.00 ± 0.77	10.00* ± 4.00	44
4.	Fraction 3 5 mg / kg in PG i / p	6	5	1	83	5.50 ± 0.90	12.00 ± 5.00	33
5.	Fraction 4 5 mg / kg in PG i / p	6	5	1	83	2.60 ± 0.76	15.26 ± 2.60	11
6.	Fraction 5 5 mg / kg in PG i / p	6	5	1	83	3.50 ± 1.00	13.00 ± 4.00	27

Levels of significance compared to control *P< 0.05; **P< 0.005 vs ASA Control, Studen's t-test.

Table 3

Effect of *Artemisia absinthium* L. extracts on mucin activity of gastric juice in untreated control and ASA treated, pylorus ligated rats

S.No.	Treatment (g / kg x 3 days P.O.)	N	Carbohydrates (µg / ml)					Total Protein (µg / ml)	Total Carbohydrates / Total Protein
			Total hexoses	Hexosamine	Fucose	Sialic acid	Total		
1.	Control	10	1312 ± 104	520 ± 72	180 ± 95	159 ± 20	2236 ± 193	2485 ± 188	0.90 ± 0.06
2.	Fraction 1 (2.0 b.i.d.)	8	1298 ± 132	250* ± 2.00	285* ± 21	158 ± 42	2399 ± 26	6000* ± 900	0.41* ± 0.075
3.	ASA (0.2 o.i.d.)	8	813* ± 69	438 ± 52.00	29 ± 5.0	164 ± 15	1443 ± 93	2000 ± 470	0.75 ± 0.090
4.	Fraction 2 (2.0 b.i.d)	6	900 ± 30	250 ± 12.00	200** ± 50	170 ± 80	1520 ± 80	4000* ± 430	0.38 ± 0.020

Results are means ± S.E. *P<0.05 vs untreated control **P<0.001 vs ASA control: Student's t-test; o.i.d = once daily; b.i.d = twice daily.

to the method of Rigges and Stadie (1933) with some modifications, as 50 ml of distilled water was used instead of 10 ml. Mercuric chloride was used as a preservative in place of thiomersalate. Significant effects have been observed in ulcer activity. The 65.00% reduction in ulcer index with fraction-I and 44.00% with fraction - II has been observed (Table 2). A qualitative change in the contents of carbohydrates (hexose and fucose) has been observed (Table 3). The extracts caused a decrease in acid and pepsin output and a qualitative change in hexose and fucose contents of carbohydrates in ASA-ulcerated rats, although the drug did not exert a quantitative change in the dissolved mucin contents of the gastric juice. The effects on swimming performance of rats have been observed (Table 4). Significant increase in the activity has been

Table 4
Effect of *Artemisia absinthium* L. fractions on swimming performance of rats

S.No.	Group	Drug treatment	N	Mean duration of swimming (Seconds ± S.E.)
1.	Control	Propylene Glycol 5 mg / kg	6	270 ± 11
2.	Fraction 1	5 mg / kg in PG i/p	5	363* ± 68
3.	Fraction 2	5 mg / kg in PG i / p	6	257 ± 23
4.	Fraction 3	5 mg / kg in PG i / p	6	255 ± 15
5.	Fraction 4	5 mg / kg in PG i / p	6	270 ± 36
6.	Fraction 5	5 mg / kg in PG i / p	5	240 ± 12

Note: *Levels of significance compared to control P<0.05 P.G. = Propylene Glycol, i/p = intraperitoneally.

observed in all fractions, especially with fraction - I and fraction - IV as compared to the controlled conditions.

LD_{50} values were not recorded as the extracts had no lethal effects upto 10 mg / kg and mortality has not been observed in the experimental animals. Therefore, it is concluded that different crude extracts of *Artemisia absinthium* have displayed significant antiulcer effects, decrease in volume of gastric juice and acid output. Injurious or toxic effects were not detected.

References

Al Habbal M J, Al Habbal Z, Huwez F W 1984 *Proceedings of the 3rd International Conference on Islamic Medicine*, Istanbul, Turkey, p 105.

Ayala W, Moore L V, Hess H J 1951 Purple colour reaction given by diphenyl amine reagent (1). *J Clin Investigation* **30** 781.

Blum A L 1985 Sources of biliary cholesterol effect. *Am J Med* **79** 8.

Dische Z, Shettles L B 1948 A specific colour reaction of methyl pentoses and a spectrophotometric micromethod for their determination. *J Biol Chem* **175** 595.

Elliot R C, Heward G J F 1976 Nitrogen intake and utilization by dairy calves. Determination of endogenous urinary nitrogen excretion by calves offered a low - nitrogen diet. *Pharm Res Comm* **8** 187.

Elson L A, Morgan W T J 1933 Colorimetric method for the determination of glucosamine and condrosamine. *Biochem J* **27** 1824.

Geol R K, Chakarbarti A, Sanyal A K 1985a *In vitro* and *In vivo* assessment of the antimalarial activity of sergeolide. *Planta Medica* **51** 2.

Geol R K, Chakarbarti A, Sanyal A K 1985b Prevention of platelet deposition by ibuprofen and calcium dobesilate in expended polytetrafluoroethylene vascular grafts. *Planta Medica* **51** 85.

Geol R K, Gouinda Das D, Sanyal A K 1985c Investigations on the formation of rubidium dimolybdate via thermal decomposition of rubidium oxomolybdenum (IV) oxalate. *Ind J Gastroenterology* **4** 249.

Geol R K, Gupta S, Shankar R, Sanyal A K 1986 The possible effect of high concentrations of propylene glycol on mitosis in human lymphocytes *in vitro*. *J Ethnopharmacology* **18** 33.

Ikram M, Shafi N, Mir I, Do M N, Nguyen P, Quesne P W Le 1987 24 - Ethylcholesta - 7; 22 Dien - 3b - ol: A possibly antipyretic constituent of *Artimesia absinthium*. *Planta Medica* **53** 389.

Lorincz A L 1994 Identification and characterization of the human and mouse promoter from a neuronal gene that maps adjacent to the Huntengtons disease gene region. *Arch Dermatol* **130** 781 - 782.

Lusting B, Langer A 1931 Determination and content of free protein bound and non - protein bound sugar in serum, cerebrospinal fluid and urine. *Biochem J* **242** 329.

Manjunath B L 1948 *Artimesia* Linn. (Compositae) *The Wealth of India, Raw Materials*; CSIR, New Delhi, India.

Okaba S, Takeuchi K, Murata T, Urushidani T 1978 Experimental animals for the study of peptic ulcers. *Am J Dig Dis* **23** 166.

Oser B L 1965 Toxicological studies with branched and linear alkylbenzenesulfoneles In: *Hawks Physioloical Chemistry*. 14th ed, Mc Graw Hill, New York, USA, pp 1131.

Priest D 1995 Leucovorin as a prodrug. *SOFW J* **121**(7) 486, 488 - 489.

Rigges B C, Stadie W C 1933 Antirachitic effect of irradiating various sized areas of skin with ultraviolet light. *J Biol Chem* **150** 463.

Rowe S J, Staton J A 1998 The neurotransmitter noradrenaline drives noggin - expressing ectoderm cells to activate N - tubulin and become neurons. In *Proceedings on NZSCC / ASCC Australasian Conference* March 1997, pp 1 - 7.

Sanyal A K, Gupta K K, Chowdhury N K 1964 Peptic ulceration (1) role of banana in phenyl butazone - induced ulcers. *Arch Intern Pharmacodyn* **149** 393.

Sanyal A K, Benerjee C R, Das P K 1965 Pharmacological studies of the total alkaloidal extract of the leaves. *Arch Intern Pharmacodyn* **155** 244.

Sanyal A K, Debnath P K, Bhattacharya S K, Gode K D 1971 Free D - amino acids in the cell pool of fungi and actinomycytes. *An Experimental Study*, Pfeiffer C J ed, Munks garrd, Copenhagen, Denmark, pp 312.

Sanyal A K, Pandey B L, Geol R K 1982 Low cost jute strike pulp from mini paper plants. *J Ethnopharmacol* **5** 79.

Shay H, Komarov S A, Fels S S, Meranze D, Gruenstein M, Siplet H 1945 Gastric mucigen action of the alkyl sulfates. *Gastroentrology* **5** 43.

Shalita A R 1995 *In vitro* effect of ciprofloxacin and afloxacin on murin and human colon carcinoma cell lines. *Int J Dermatol* **34**(6) 434 - 437.

Siddiqui A A, Ali M 1997 Identification tests of organic drugs. In: *Practical Pharmaceutical Chemistry* Ist ed, CBS Publishers and Distributors. New Delhi, India, pp 120 - 132.

Sinsaku N, Nobuo I, Makoto S 1981 Detection and isolation of steriod saponins In: *Advances in Natural Products Chemistry*. John Wiley & Sons, New York - Chichester - Brisbane - Toronto, pp 292 - 309.

Winzler R J, Dener A W, Mehl J W, Smyth I M 1948 Isolation and characterization of proteose from human plasma. *J Clin Investigation* **27** 609.