Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

EFFECT OF DIFFERENT SOLVENT EXTRACTS OF *FUMARIA VAILLANTII* L. ON EXPERIMENTAL HYPOCHLORHYDRIA IN RAT

UPANANDAN MANDAL[a], DILIP KUMAR NANDI[b], KAUSIK CHATTERJEE[a], KAZI MONJUR ALI [a], ANJAN BISWAS[c] DEBIDAS GHOSH [a,*]

[a]Andrology, Endocrinology & Molecular Medicine Laboratory, Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore-721102, West Bengal, India, [b]Department of Physiology, Raja N.L. Khan Women's College, Midnapore-721 102, West Bengal, India, [c]Department of Physiology, Presidency University, Kolkata, West Bengal, India. Email: debidasghosh60@gmail.com

ABSTRACT

The aim of the present study was undertaken to evaluate the effects of the different solvent extracts of whole plant of *Fumaria vaillantii* Loisel on gastric hyposecretion in experimental condition in albino rats. Hypochlorhydria was induced in healthy rat by orally administration of ranitidine at the dose of 5 mg/kg body weight in alternative day for14 days. We used three solvent extracts of whole plant of *Fumaria vaillantii* L. as HMFV (Hydro-methanol extract of *Fumaria vaillantii* L), EAFV (Ethyl acetate extract of *Fumaria vaillantii* L.) and AFV (Aqueous extract of *Fumaria vaillantii* L.) for searching the most effective extract for the correction of hypochlorhydria. It was found that pre-administration followed by co-administration of AFV prevents the ranitidine induced hypochlorhydria and increased the antioxidant status in gastric tissue along with vitamin C, pepsin and chloride levels in gastric juice in rat more significantly than other extracts used here. It has been concluded that AFV is an effective anti-hypochlorhydric and anti-lipidperoxidative agent with respect to other two extract of said plant.

Keywords: Hypochlorhydria, Gastric pH, Vitamin C, Ranitidine, *Fumaria vaillantii* L.

INTRODUCTION

Hypochlorhydria stands for low secretion of hydrochloric acid by the gastric cells and increased the intragastric pH (pH ≥ 4). Low gastric acid provides the suitable media for colonization of different types of bacteria in the stomach[1, 2]. Hypochlorhydria is a very common problem and leads to a number of digestive complaints including *Helicobacter pylori* infection, bowel toxemia, dysbiosis, pancreatic insufficiency and leaky gut syndrome. Some common signs and symptoms of low gastric acid are bloating, belching, burning and flatulence immediately after meals, a sense of 'fullness' after eating, indigestion, diarrhea, constipation etc. Beside these, others are multiple food allergies and nausea, itching around the rectum, acne, iron deficiency, chronic intestinal parasites and abnormal flora, indigested food in stool and chronic candida infection[3, 4]. Hypochlorhydria has a so many number of etiologies such as sympathetic dominance, antiseretory drug use, excess sugar and refined foods, chronic over eating, constant smoking between meals and nutrient deficiencies especially zinc and thiamin. In hypochlorhydric condition vitamin C concentration is lowered in gastric juice [5]. Vitamin C inhibits the formation of carcinogenic N- nitroso compounds with in the gastric juice of healthy stomach [6, 7]. So hypochlorhydria is a risk factor of gastric cancer. Stress initially increases stomach HCl production and causes indigestion, heartburn, gastritis and ulcer problems. But chronic stress can lead to hypochlorhydria and reduced function of the pancreas [8].

In this present study hypochlorhydria in rat was induced by the orally administration of ranitidine, a H_2 receptor antagonist with a standard dose [9]. In our earlier work it was reported that *Fumaria vaillantii* Loisel (*Fumaria vaillantii* L.) has anti-hypochlorhydric activity that protects the experimental hypochlorhydria and age induced hypochlorhydria in rat [9, 10]. *Fumaria vaillantii* L. belongs to family of Fumariaceae (fumitory). Its local name is 'Parpata' or 'Pitpapra' or 'Parpatakam [11]. It is found in India, Pakistan, Afghanistan, Central Asia, North Dakota and Colorado. The whole plant is used for the treatment of constipation, diarrhoea, amlapitta, hypochlohydria and liver complications [9, 12, 13]. *Fumaria vaillantii* L. is widely used in folk medicine as a blood purifier in the treatment of skin diseases [14]. Therefore, the aim of the present study is to search out the effective solvent extract of this plant for the management of hypochlorhydria.

MATERIALS AND METHODS

Plant materials

The whole plants of *Fumaria vaillantii* L. (FV) were collected from sub Himalayan region (India) in the month of September. Whole plant of *Fumaria vaillantii* L. was identified and preserved in Botany Department, Vidyasagar University, West Bengal, India [9]. The whole plant of *Fumaria vaillantii* L. was air dried and powdered finely by grinding and then stored in air tight vessels as reported previously [9].

Hydro-methanol extract of *Fumaria vaillantii* L. (HMFV)

Twenty five gram powder of whole plant of *Fumaria vaillantii* L. was macerated with 150 ml of aqueous-methanol (2: 3, v: v) mixture at 37 ^{0}C for 36 hrs with intermittent stirring. Later, the extract was filtered and filtrate was dried by low pressure and residue was collected. This residue was suspended in distilled water at a concentration of 4 mg/ml to be used for the experiment.

Ethyl acetate extract of *Fumaria vaillantii* L. (EAFV)

Twenty five gram powder of whole plant of *Fumaria vaillantii* L. was macerated with 150 ml of ethyl-acetate at 37 ^{0}C for 36 hrs with intermittent stirring. Later, the extract was filtered and filtrate was dried by low pressure and residue was collected. This residue was suspended in distilled water at a concentration of 4 mg/ml to be used for the experiment.

Aqueous extract of *Fumaria vaillantii* L. (AFV)

Twenty five gram powder of whole plant of *Fumaria vaillantii* L. was macerated with 150 ml of distilled water at 37 ^{0}C for 36 hrs with intermittent stirring. Later, the extract was filtered and filtrate was dried followed by collection as powdered form. This residue was suspended in distilled water at a concentration of 4 mg/ml to be used for the experiment as reported previously [9].

Chemicals

All chemicals were analytical grade and were purchased from E. Merck and Loba (India).

Animals

Thirty male Wistar strain young (3 month old) albino rats having weight 100 ± 5 gm were selected for this experiment. The rats were acclimatized for a period of 15 days in our laboratory prior to the experiment. All studies were conducted in accordance with the

National Institute of Health's Guide for the care and use of Laboratory animals. The work was approved by our University Ethical committee. Animals were housed at an ambient temperature of 25 ± 2 ^{0}C with 12 hr light: 12 hr dark cycle. Animals were given free access to water and food.

Induction of hypochlorhydria

Hypochlorhydria was induced in healthy rat by orally administration of ranitidine at the dose of 5 mg/kg body weight in alternative day for14 days as our earlier work [9].

Experimental design

Animals were divided into following five groups containing six rats in each group and duration of the experiment was of 16 days (2 days pre-treatment and 14 days co-treatment). All the drugs were administered in oral route by gavages.

Group I (Control group)

Animals received only distilled water (0.5 ml / 100 g).

Group II (Hypochlorhydric group or ranitidine treated group)

Rats were given distilled water for two days then treated with ranitidine at a dose of 5 mg/kg of body weight in alternative day before meal for 7 such doses.

Group III (HMFV pretreatment cum co-treatment group)

Rats of this group received HMFV at a dose of 20 mg/kg body weight/day for two days followed by the treatment of ranitidine at the dose of 5 mg/kg body weight in alternative day along with treatment of the same extract once a day before meal as above mention dose up to the end of the experiment (14 days of co-treatment).

Group IV (EAFV pretreatment cum co-treatment group)

Rats of this group received EAFV at a dose of 20 mg/kg body weight/day for two days followed by the treatment of ranitidine at the dose of 5 mg/kg body weight in alternative day along with treatment of the same extract once a day before meal as above mention dose up to the end of the experiment (14 days of co-treatment).

Group V (AFV pretreatment cum co-treatment group)

Rats of this group received AFV at a dose of 20 mg/kg body weight/day for two days followed by the treatment of ranitidine at the dose of 5 mg/kg body weight in alternative day along with treatment of the same extract once a day before meal as above mention dose up to the end of the experiment (14 days of co-treatment).

Gastric juice collection

The animals of all the groups were fasted for 24 hrs after completion of above treatment schedule. Under light ether anesthesia, the abdomen was opened through midline incision and the pylorus was ligated [15]. The stomach was placed in the usual position and then abdomen was sutured. Four hour later, pylorus ligated rats were sacrificed with anesthetic ether. After opening the abdomen, the esophagus was clamped and then stomach was removed. The gastric juice was collected and its volume was measured followed by centrifugation. Liver and kidney from each animal were collected and their wet weights were noted and stomach was collected for biochemical estimation.

Measurement of pH of gastric juice

The pH of the gastric juice was measured by using pH meter. Hypochlorhydria was defined as a fasting gastric pH > 4.0.

Estimation of free acidity and total acidity

The free acidity (free HCl) of the gastric juice was estimated by titration [16] with N/10 NaOH solution using Topfer's reagents (0.5 gm diethyl amino azobenzenes /100 ml ethanol) as an indicator.

Total acidity includes free acids, hydrochloric acid combined with protein, and organic acid and acid salts. It was estimated by titration with N/10 NaOH solution using phenolphthalein as an indicator[16].

Quantification of chloride level in gastric juice

At first protein free filtrate of gastric juice was prepared. In brief, chloride level was measured in gastric juice by diluting with water followed by mixing with sodium tungstate and H_2SO_4. The mixture was centrifuged and protein free filtrate was collected. This filtrate was titrated against mercuric nitrate solution using diphenyl-carbazone as an indicator [17].

Assessment of pepsin concentration in gastric juice

The pepsin in gastric juice was estimated by the method of Smuual Natelson [18]. In brief, gastric juice was incubated with pepsin substrate (0.5% bovine haemoglobin) and centrifuged. Then supernatant was treated with Folin phenol reagent and absorbance was measured spectrophotometrically at 540 nm wave length.

Measurement of vitamin-C level in gastric juice

Vitamin-C level was measured using the 2, 4-dinitrophenyl hydrazine method [19, 20]. Two ml of 10% metaphosphoric acid was added to 0.5 ml of plasma or gastric juice to precipitate protein. After vortex mixing, samples were centrifuged at 900 g for 10 minutes and filtered through a 0.45 µm filter paper. Next, 1.2 ml of the filtered was mixed with 0.4 ml reaction buffer (5 ml 27 µmol/L copper sulphate, 5 ml 660 µmol/L thiourea and 10 µmol/L 2, 4 dinitrophenylhydrazine).

The mixture was vortexed and stored in water bath at 37 ^{0}C for 3 hrs. The samples were then placed in ice for 10 minutes and were added to 2 ml of 12 mol/L H_2SO_4 carefully. The absorbances of samples were measured spectrophotometrically at 520 nm wave length. Ten percent metaphosphoric acid was used as blank and 1 mg/dl ascorbic acid was measured as a standard.

Estimation of lipid peroxidation in stomach

Lipid peroxidation in stomach was assessed by measuring the concentration of thiobarbituric acid-reactive substances. The gastric tissue was homogenized to 50 mg /ml in 0.1 M of ice-cold phosphate buffer (pH 7.4) and the homogenate was centrifuged. The 0.5 ml of supernatant was mixed with 0.5 ml of normal saline and 2 ml of TBA-TCA (0.392 g Thiobarbituric acid in 75 ml of 0.25 N HCl with 15 g trichloro acetic acid) mixture. The volume of mixture was made up to 100 ml with 95 % ethanol and boiled at 100^{0}C for 10 minutes. This mixture was then cooled to room temperature and centrifuged at 4000 X g for 10 minutes. The whole supernatant was taken into a spectrophotometer cuvette and read at 535 nm [21].

Biochemical assay of catalase activity of gastric tissue

The activity of catalase in gastric tissue was measured biochemically [22]. For the evaluation of catalase activity, gastric tissue was homogenized in 0.05 M Tris-HCl buffer solutions (pH 7.0) to a concentration of 50 mg/ml. These homogenized samples were centrifuged at 10000 X g at 4^{0}C for 10 minutes. In spectrophotometer cuvette, 0.5 ml of 0.00035 M H_2O_2 and 2.5 ml of distilled water were added and mixed. Readings of absorbance were noted at 240 nm before the addition of supernatant. Supernatant from the sample was added at a volume of 40 µl to the cuvette and the subsequent six readings were noted at 30 sec interval.

Statistical analysis

Data were reported as means ± SEM. ANOVA followed by a multiple comparison two tail't' test was used for statistical analysis of the collected data. Differences were considered significant when $p<0.05$.

Table 1: Protective effect of pre-treatment followed by co-treatment of three different solvent extracts of whole plant of *Fumaria vaillantii* L. on body weight, hepato-somatic indices and reno-somatic indices in ranitidine induced hypochlorhydric group and extract pre-treated cum co-treated groups.

Groups	Body Weight (gm)		Hepato-somatic index (gm/100 gm body weight)	Reno-somatic Index (gm/100 gm body weight)
	Initial	Final		
Control	100.2 ± 5.02.[a]	100 ± 4.62 [a]	2.83 ± 0.42[a]	0.68 ± 0.04 [a]
Ranitidine treated	104.2 ± 4.02 [a]	103.4 ± 4.43 [a]	2.65 ± 0.43 [a]	0.65 ± 0.03 [a]
HMFV	103.7 ± 5.63 [a]	97.5 ± 4.47 [a]	2.70 ± 0.46 [a]	0.67± 0.04 [a]
EAFV	103.5 ± 4.75 [a]	100.4 ± 5.26 [a]	2.75 ± 0.32 [a]	0.63 ± 0.05[a]
AFV	100.2 ± 5.62 [a]	98.5 ± 5.13 [a]	2.81 ± 0.25 [a]	0.64 ± 0.04 [a]

Data are expressed as mean ± SEM, n=6. Values with same superscript (a) in each vertical column do not differ significantly from other (p>0.05). ANOVA followed by multiple comparison two-tail "t" test.

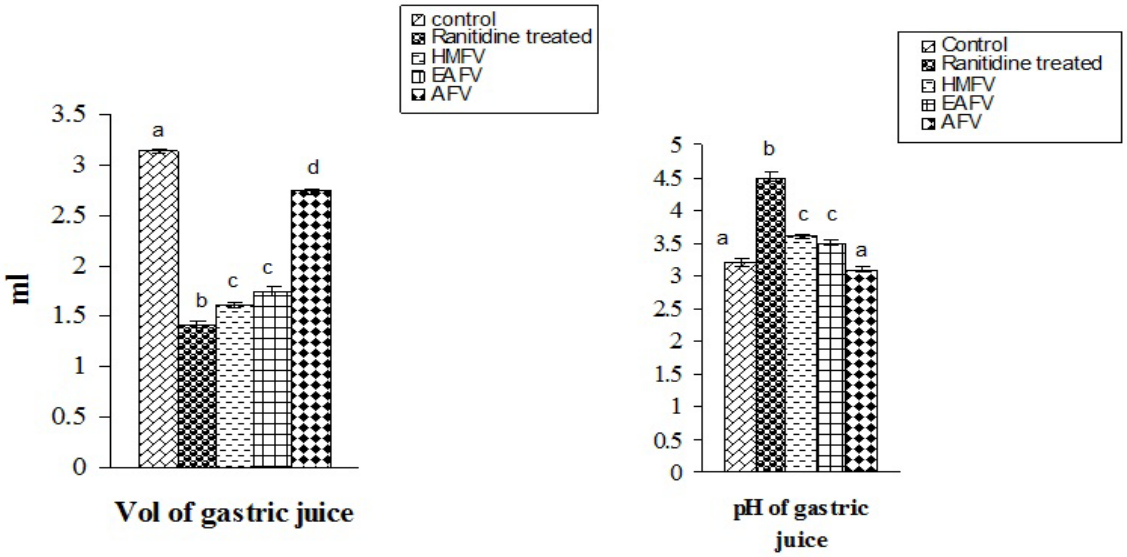

Fig. 1: Effect of pre-administration followed by co-administration of HMFV or EAFV or AFV on volume and pH of gastric secretion in ranitidine induced hypochlorhydric rat.

Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) significantly differ from each other (P< 0.05). ANOVA followed by multiple comparison two-tail "t" test.

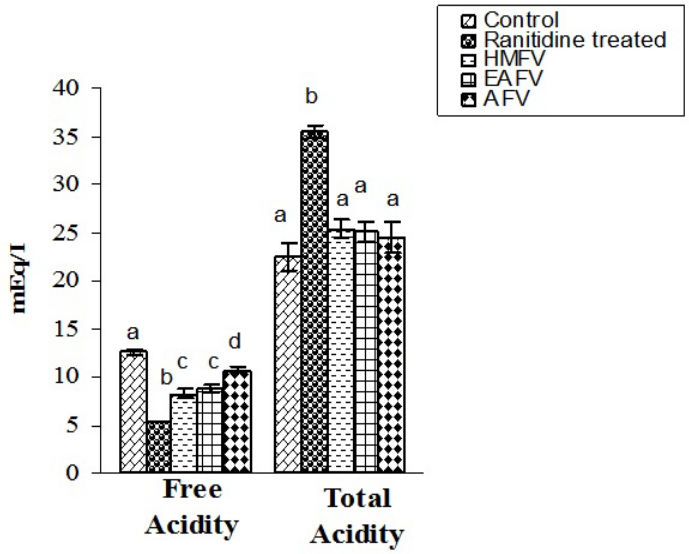

Fig. 2: Corrective effect of pre-treatment followed by co-treatment of HMFV or EAFV or AFV in free acidity and total acidity of gastric secretion in ranitidine induced hypochlorhydric rat.

Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) significantly differ from each other (p < 0.05). ANOVA followed by multiple comparison two-tail "t" test.

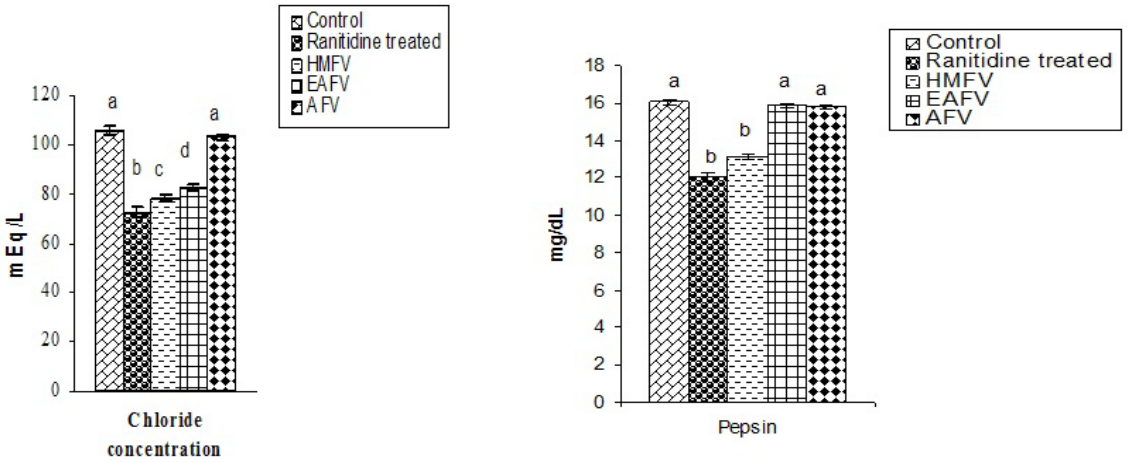

Fig. 3: Effect of pre-administration followed by co-administration of HMFV or EAFV or AFV on chloride and pepsin concentration in ranitidine-induced hypochlorhydric rat.

Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) significantly differ from each other ($p < 0.05$). ANOVA followed by multiple comparisons two-tail "t" test.

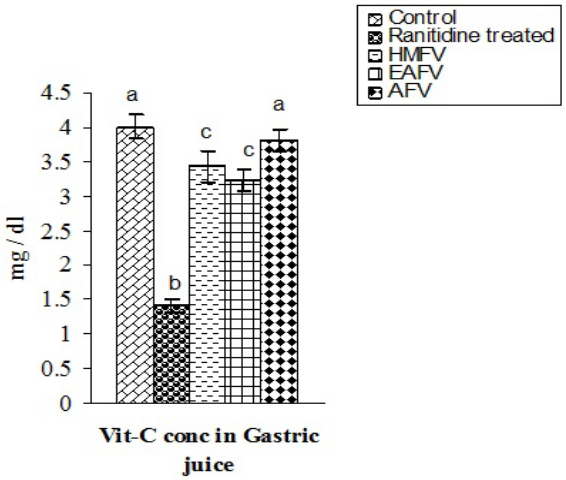

Fig. 4: Protective effect of pre-administration cum co-administration of HMFV or EAFV or AFV on vitamin C concentration in gastric juice in ranitidine induced hypochlorhydric rat.

Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c) significantly differ from each other ($P< 0.05$). ANOVA followed by multiple comparisons two-tail "t" tests.

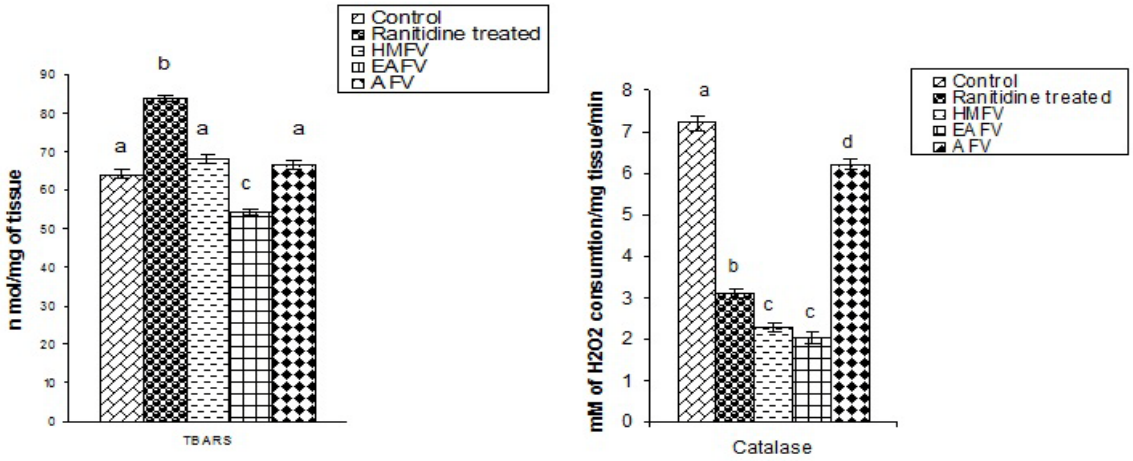

Fig. 5: Remedial effect of pre-administration followed by co-administration of HMFV or EAFV or AFV on TBARS level and catalase activity in gastric tissue in ranitidine-induced hypochlorhydric rat.

Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) significantly differ from each other ($p < 0.05$). ANOVA followed by multiple comparisons two-tail "t" test.

RESULTS

Body weight and organo-somatic indices

In this present study there was no significant difference in body weight among the five groups. No significant alteration was noted in hepato-somatic and reno-somatic indices in ranitidine-treated and extract of *Fumaria vaillantii* L. pre-treatment cum co-treatment groups in compare with the control group (Table I).

Volume of gastric juice

Volume of basal gastric secretion was decreased significantly in ranitidine-induced hypochlorhydric rat in comparison with the control. Pre-treatment followed by co-treatment of three different extracts of *Fumaria vaillantii* L (HMFV or EAFV or AFV) to ranitidine treated rat increased the volume of gastric secretion significantly when compare to only ranitidine-treated rat. But pre-treatment followed by co-treatment of AFV to ranitidine treated rat, the level of this parameter reached toward the control level (Figure 1).

Gastric juice pH

Gastric juice pH was increased significantly in ranitidine-induced hypochlorhydric group with respect to the control group. Pre-treatment followed by co-treatment of HMFV or EAFV or AFV decreased the gastric pH in compare to ranitidine treated group. Significant recovery was noted in this parameter, in AFV pre-treated cum co-treated group in respect to other two solvent extract treated groups (Figure 1).

Free acidity and total acidity

It was found that the level of free acidity was decreased significantly and total acidity was increased significantly in ranitidine-induced hypochlorhydric group in comparison with the control group. Pre-administration as well as co-administration of HMFV or EAFV or AFV resulted a significant elevation in free acidity in compare with the ranitidine-treated group and significant reduction was noted in total acidity with respect to ranitidine induced hypochlorhydric group. Here, AFV showed more effective response to restore the acidity of the stomach towards the control level (Figure 2).

Chloride level in gastric juice

Chloride secretion was decreased significantly in ranitidine-induced hypochlorhydric rat in comparison to the control group. Pre-treatment followed by co-treatment of HMFV or EAFV or AFV to ranitidine treated rat increased the level of chloride in gastric secretion significantly when compare to ranitidine-induced hypochlorhydric group. But AFV pre-treatment followed by co-treatment recovered the chloride level in gastric juice with respect to other pre-treated cum co-treated groups (Figure 3).

Pepsin concentration in gastric juice

Pepsin activity was decreased significantly in ranitidine-induced hypochlorhydric rat in comparison to the control group. Pre-treatment followed by co-treatment of HMFV or EAFV or AFV to ranitidine treated rat increased the pepsin activity significantly when compare to ranitidine-induced hypochlorhydric group. Pre-treatment followed by co-treatment of EAFV resets this parameter to control though no significant difference was noted between EAFV and AFV pre-treatment cum co-treatment groups (Figure 3)

Vitamin C concentration in gastric juice

A significant depletion was noted in vitamin C concentration in gastric juice in ranitidine-induced hypochlorhydric group when compare with the control group. Pre-treatment followed by co-treatment of HMFV or EAFV significantly increased the level of vitamin C in gastric juice but not reached to the control level. Pre-treatment cum co-treatment of AFV to ranitidine-treated rat resulted a significant elevation in vitamin C concentration which reached towards the control level (Figure 4).

Thiobarbituric acid-reactive substances (TBARS) levels in gastric tissue

Gastric tissue TBARS level was increased significantly in ranitidine-induced hypochlorhydric group with respect to the control group. Pre-treatment followed by co-treatment of HMFV or EAFV or AFV decreased the gastric TBARS in compare to ranitidine treated group. More effective response was noted in this parameter, in EAFV pre-treated cum co-treated group in respect to other two solvent extract treated groups (Figure 5).

Activity of catalase in gastric tissue

A significant depletion was noted in gastric tissue antioxidant enzyme i.e catalase activity in ranitidine-induced hypochlorhydric group when compare with the control group. Pre-treatment followed by co-treatment of HMFV or EAFV do not gave satisfactory result for the restoring this parameter. Pre-treatment cum co-treatment of AFV to ranitidine-treated rat resulted a significant elevation in catalase activity which reached towards the control level (Figure 5).

DISCUSSION

The present study was conducted to find out the effective extract having anti hypochlorhydric activity among the different solvent extracts of whole plant of *Fumaria vaillantii* L. such as HMFV, EAFV and AFV. We have found that there were no significant differences in body weight gain among the different groups and no significant difference was observed in food ingestion and water intake behavior throughout the experimental period. So, it can be said that the above herbal extracts have no general metabolic toxicity. Several studies have shown that the ability of gastric acid secretion is decreased with advancement of age and it becomes half of those over age 60 years [23, 24]. The incidence of hypochlorhydria in the population has been estimated about 20-50 % (in average 30 % of the population) above 65 years age group [25, 26]. Now it's a common problem of people of the modern age due to frequently and unnecessary use of anti - secretory drugs such as histamine receptor antagonist e.g. ranitidine and proton pump inhibitors e.g. omeprazole by self-doctoring. These drugs suppress the acid secretion in stomach resulting hypochlorhydria [27, 28] defined as a fasting gastric pH above 4.0 [5]. Normally, the resting stomach contains appreciable amount of free acid and it maintains the pH of the stomach [29]. Use of histamine H_2 receptor antagonist like ranitidine raises this intra gastric pH [30]. From our current studies, we found that HMFV or EAFV or AFV increased the basal volume of the gastric juice and decreased the pH of gastric juice by increasing the free acidity and chloride secretion in the gastric juice, supported by our earlier work [9]. Here, we noted that gastric secretory activity was restored after pre-administration as well as co-administration of AFV to ranitidine treated rat but other two solvent extracts of said plant do not gave satisfactory results for restoring the gastric secretory activity from hypochlorhydric state. This may be due to the prevention of the gastric parietal cell degeneration or by stimulating the secretion of HCl via stimulating cholinergic parietal cell stimulation, supported by our earlier work and others [9, 10, 31]. It was reported that the *Fumaria vaillantii* L. has anti acetylcholine esterase activity [31] and thus it stimulates the parietal cells. We also observed that pepsin activity and chloride level were decreased in gastric juice of rats treated with ranitidine in alternate day for 14 days. This findings supported by others work [32, 33]. There are two components of the luminal chloride secreted by the parietal cells, the acidic component of chloride secretion, which is essential for gastric hydrochloric acid secretion, and non-acidic component, which is observed as a transmucosal movement of chloride in excess of hydrogen [34, 35]. The pre-treatment followed by co-treatment of AFV increased the chloride level significantly in compare to ranitidine treated group and other extract treated groups. This may stimulate the parietal cell by the active ingredients of AFV. We also found that gastric antioxidant such as vitamin C in gastric juice and catalase activity of gastric tissue were decreased in ranitidine induced hypochlorhydric rat in compared to control. Moreover products of lipid peroxidation such as TBARS level also increased in hypochlorhydric group with respect to control supported by other [5, 36, 37]. These findings suggest that hypochlorhydria may causing oxidative stress which producing free radicals. So what catalase and vitamin C were used to scavenging free radicals, resulting the diminishing those parameters in hypochlorhydric group. In this present study, we found that HMFV or EAFV or AFV extracts increased the gastric secretion along

with vitamin C concentration in the gastric juice and they may act as the secretagogues on the stomach supported by our earlier work [9]. For the recovery of vitamin C level, AFV gave more satisfactory results than HMFV or EAFV. AFV also able to reduce the level of TBARS significantly in compare to ranitidine treated rat but not reached to the control level. EAFV pre-treated cum co-treated group reached to the control level and catalase activity reached towards the control level in AFV pre-treated cum co-treated group. These findings suggest that *Fumaria vaillantii* L. has anti-lipidperoxidative and antioxidant property reported by our earlier work and other [10, 38]. Therefore, it may be concluded that AFV (aqueous extract of whole plant of *Fumaria vaillantii* L.) has more potent antihypochlorhydric activity and also possess antioxidant property and the effects may be attributed to the components such as alkaloids and antioxidant principles present in the AFV.

ACKNOWLEDGEMENT

The authors would like to acknowledge to University Grants Commission for providing financial support.

REFERENCES

1. Modlin IN, Sachs G. Acid related diseases. In: Biology and treatment, Schnetztor Verlag GmbH. DKonstanz (Eds) 1998; p197-241.
2. Naylor G, Aoxn A. Role of bacterial overgrowth in the stomach as an additional risk factor for gastritis. Canadian J of Gastroenterol 2003; 17: 13B-17B.
3. Wright JV. Wright's guide to healing with nutrition. New Canaan, CT: Keats Publishing 1985; 190: 155.
4. Kanno T, Mastuki T, Oka M. Gastric acid reduction leads to an alteration in lower intestinal microflora. Biochem and Biophy Res Comm 2009; 381: 666-670.
5. O'Connor HJ, Schorah CJ, Habibzedah N, Axon ATR, Cockel R. Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease and possible sources. Gut 1989; 30: 436-442.
6. Mirvish SS, Wallcave L, Eagen M, Shubik P. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science (Wash) 1972; 177: 65-68.
7. Kyrotopoulos SA. Ascorbic acid and the formation N-nitroso compoundds: possible role of ascorbic acid in cancer prevention. Am J Clin Nutr 1987; 45: 1344-1350.
8. Hass ME, Levin B. Staying healthy with nutrition. 21st Century edition; p 600.
9. Mandal U, Nandi D, Chatterjee K, Biswas A, Ghosh D. Remedial effect of aqueous extract of whole plant of *Fumaria vaillantii* Loisel and ripe fruit of *Benincasa hispida* Thunb in ranitidine induced-hypochlorhydric male rat. Int J Appl Res Nat Prod 2010; 3: 37-47.
10. Mandal U, De D, Nandi DK, Biswas A, Ghosh D. Anti-hypochlorhydric and antilipidperoxidative activity of composite extract of whole plant of *Fumaria vaillatii* and ripe fruit of *Benincasa hispida* on aged male albino rat. Pharmacologyonline 2009; 1: 573-577.
11. Nadkarni KM. *Fumaria vaillantii* Loisel. In: Nadkarni KM, editor. The Indian Materia Medica. Vol. 1. 3rd Ed, Bombay, Popular Prakashan; 1993: 560.
12. Gilani AH, Basir S, Janbaz KH, Khan A. Pharmacological basis for the use of *Fumaria indica* in constipation and diarrhea. J Ethnopharmacol 2005; 15: 585-589.
13. Reza Mortazavi, Nassiri-Asl M, Farahani-Nick Z, Savad S, Kamal Farzam S. Protective effects of *Fumaria vaillantii* extract on carbon tetrachloride induced hepatotoxicity in rats. Pharmacologynline 2007; 3:385-393.
14. Sener B, Oran I. Discovery of drug candidates from some Turkish plants and conservation of biodiversity. Pure and Appl Chem 2005; 77: 53-54.
15. Shay H, Komarov SA, Feles SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945; 5: 43-61.
16. Sengupta J. In: Synopsis of clinical pathology and Microbiology. Hilton and Company, Calcutta. India, 5th ed. p. 120.
17. Nath RL. Practical biochemistry in clinical Medicine. Academic Publisher. Calcutta. India. 2nd Ed. 1990; p. 280.
18. Natelson S. Technique in clinical chemistry. Chals C Thomas Publisher. USA. 3rd ed. 1971; p. 541.
19. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dihydro-ascorbic acid. J Biol Chem 1943; 147:399-07.
20. Park HJ, Kim YS, Kim WD, Lee GW, Rhee HK, Youn SH. Correlation between *Helicobacter pylori* infection and vitamin C levels in whole blood, plasma and gastric juice and pH of Gastric juice in Korean children. J Pediatric Gastroenterol Nut 2003; 37: 53-62.
21. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues thiobarbituric acid reaction. Annals of Biochem 1979; 95: 351-358.
22. Beer RF, Sizer IW. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 195: 133-140.
23. Rafsky HA, Weingarten M. A study of the gastric secretory response in aged. Gastroenterology 1946; 8: 348-352.
24. Krasinski SD, Russell RM, Samloff IM. Fundic atrophic gastritis in an elderly population. Effect on hemoglobin and several serum nutritional indicators. J Am Geriatric Soc 1986; 34: 800-806.
25. Wolters M, Strohle A, Hahn A. Cobalamine: a critical vitamin in the elderly. Preview of Med 2004; 39: 1256-1266.
26. Plummer N. The unseen epidemic: the linked syndromes of achlorhydria and atrophic gastritis. Townsend Letter for Doctors and Patients. 2004.
27. Gledhill T, Leicester RJ, Addis B, Lightfoot N, Barnard J, Viney N, Darkin D, Hunt RN. Epidemic hypochlorhydria. British Med J 1985; 290: 785-790.
28. Belaiche J, Zitton J, Marquet J. Effect of ranitidine on gastric intrinsic factor and cobalamine absorption. Gastroenterol Clinl Biol 1983; 7: 381-384.
29. Sing P, Indaram A, Greenberg R, Visvalingam V, Bank S. Longterm omeprazole therapy for reflux esophagitis: follow-up serum gastrin levels, EC-cell hyperplasia and neoplasia. World J Gastroenterol 2000; 6: 789-792.
30. Morris AJ, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987; 82: 192-199.
31. Orhan EI, Sener B, Chudhary IM, Khalid A. Acetylcholine esterase and butyrolcholine esterase inhibitory activity of some Turkish medicinal plants. J Ethnopharmacol 2004; 91: 57-60.
32. Feldman M, Goldschmiedt M. Gastric HCO_3^- secretion: relationship with Na^+ secretion and effect of acetazolamide in humans. Am J Physiol 1991; 261: G 320-G 326.
33. Berendt WA, Zerr CH, Santa Ana CA, Porter JL, Fordtran JS. Proton-Pump Inhibition of gastric chloride secretion in congenital chloridorrhea. The New Eng J Medicine1997; 336: 106-109.
34. Machem TE, Mclennan WL. Na^+ dependent H^+ and Cl^- transport in vitro frog gastric mucosa. Am J of Physiol 1980; 238: G 403-G 413.
35. Coskum T, Baumgartner HK, Chu S, Montrose MH. Coordinated regulation of gastric chloride secretion with both acid and alkali secretion. Am J Physiol 2002; 283: G 1147-G 1155.
36. McColl KE.. Effect of proton pump inhibitors on vitamins and iron. Am J Gastroenterol 2009; 104: 5-9.
37. Parke DV. Sixty years of research into gastric cancer. Toxicol Ecotoxicol News 1997; 4: 132-137.
38. Orhan EI, Sener B, Mursharraf GS. Antioxidant and hepato protective activity appraisal of four selected Fumaria species and their total phenol and flavonoid quantities. Experimental and Toxicologic Pathol 2010; online.