

**ANTIHYPOCHLORHYDRIC AND ANTILIPIDPEROXIDATIVE EFFECT OF
COMPOSITE EXTRACT OF WHOLE PLANT OF *FUMARIA VAILLANTII*
AND RIPE FRUIT OF *BENINCASA HISPIDA* ON AGED MALE ALBINO RAT**

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Summary

The present study was designed to determine the relationship between changes in gastric function caused by aging and herbal management of aging induced changes in gastric functions. Gastric secretions decline with the advancement of age. Iron overload in the liver is associated with aging along with haemoglobin deficiency. The gastric secretion studies were undertaken using pylorus ligation model. The results of this study demonstrated that the treatment with aqueous extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* in a combined manner to the aged rats significantly increased the basal gastric secretion along with vitamin C concentration in gastric juice and reduced the gastric pH which was resulted towards the levels of the young rat. This composite extract also reduced the lipid peroxidation induced by overload of iron in liver due to aging. Thus this extract supplementation increased the serum iron along with haemoglobin percentage in aged rat. This study suggests that composite extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* is antihypochlorhydric and antilipidperoxidative which is useful for the management of age induced gastric disorders.

Key Words: Vitamin C, Aging, Gastric secretion, Liver iron, *Benincasa hispida*

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Introduction

Aging has been defined as the progressive accumulation of changes with time which are associated with ever-increasing susceptibility to disease. Aging is associated with secretory and morphologic changes in the stomach. Gastric secretions decline with advancement of age (1) and it becomes half at the age over 60 years in respect to young age (2,3). The fall in gastric secretion in older individual due to loss of parietal cells activity in the stomach (2). Hypochlorhydria is a condition where the parietal cells of the stomach secrete insufficient amount of hydrochloric acid (HCl). The fasting gastric pH is the reliable indicator of the hypochlorhydria (4, 5). Low gastric acid provides the suitable media for colonization of different types of bacteria in the stomach (6, 7). *Helicobacter pylori* infection is associated with hypochlorhydria and it is a common agent for the destruction of parietal cells (8). The proper production of HCl is essential for optimal health. It not only helps digestion but also it keeps the stomach sterile against pathogens and enables the proper absorption of protein and a variety of nutrients (9). The incidence of many gastrointestinal dysfunctions, including malignancy increases with advancement of age, which in itself is associated with alterations in the structural and functional integrity of the gastrointestinal tract (10,11). Gastric cancer rarely occurs before the age of 40 years, but its incidence increases subsequently with peak incidence occurring in the seventh decade. Many probable reasons including altered carcinogen metabolism and long-term exposure of cancer-causing agents have been offered for the age-dependent rise in malignancies (12). Vitamin C in the gastric juice acts as a antioxidant and scavenges reactive oxygen metabolites in the stomach and prevent the formation of N-nitrosocompounds that are mutagenic causes gastric cancer (13). Vitamin C content is low in the gastric juice of patients with hypochlorhydria (14). So hypochlorhydria is the risk of gastric cancer pathogenesis. Gastric cancer is the second leading cause of cancer death and the fourth most common cancer in terms of new cases worldwide (15). *H pylori* infection leads to changes in many factors, such as the vitamin C content of gastric juice, the levels of reactive oxygen metabolites in the tissues and epithelial cell proliferation, which are important in the pathogenesis of gastric cancer (16). Other age related changes are decline of haemoglobin in blood in humans and in animal models (17-22) while others have reported no age-related changes in these parameters (23-24). There is also evidence suggesting that iron store in the liver of the older animals is high (25-28). Lipid peroxidation and oxidative damages accelerate the aging (25, 27, 29, 31), and iron is a causative agent for lipid peroxidation (31). A significant increased lipid peroxidation in liver have been observed in rats (32) because of increased iron store in the liver due to aging. Excess iron causes stress in body since iron is catalyst for lipid peroxidation (31) and for reactive radicals generation (33). In normal young individual, total body iron levels are balanced by means of absorption and elimination (34) but aging affects the removal of iron from the body results in a slow accumulation of iron in the liver. In traditional medicine various herbal preparations are used for the treatment of gastrointestinal problems. Different ayurvedic texts deal with *Fumaria vaillantii* and *Benincasa hispida* as herbal medicine for the gastrointestinal diseases (35, 36).

In the absence of an effective treatment for hypochlorhydria in modern medicine without any side effects, efforts are being made of suitable herbal drug. *Fumaria vaillantii* belongs to family of 'Fumariaceae' (fumitory). Its local names are 'Parpata' or 'Pitpapra' or 'Parpatakam'(35). *Benincasa hispida* belongs to 'Cucurbitaceae' family. It is commonly known as 'Ash gourd' or 'Chalkumra' or 'Kusmanda' (36). The fruit of *Benincasa hispida* has been used in India for centuries for various ailments such as gastrointestinal problems, respiratory disease, heart diseases, diabetes mellitus and urinary diseases (37).

In this present study, we have examined the effect of composite extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* on gastric secretion in aged rat.

Materials and Methods

Plant materials: The fruits of *Benincasa hispida* were collected from the local areas in the month of June and the whole plants of *Fumaria vaillantii* were collected from sub Himalayan region (India) in the month of September. Fruits of *Benincasa hispida* and whole plant of *Fumaria vaillantii* were identified in Botany department, Vidyasagar University, West Bengal, India. The juice of the fruit was collected and stored at 4 °C. The whole plant of *Fumaria vaillantii* was air dried and powdered finely by grinding and then stored in air tight vessels.

Preparation of composite extract of *Fumaria vaillantii* and *Benincasa hispida*

At first the aqueous extracts of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* were prepared separately. Then aqueous extract of *Fumaria vaillantii* and *Benincasa hispida* were used at the ratio of 1:1 (w/w) to prepare the composite extract. The extract was prepared as per standard protocol reported earlier (38).

In brief, for the preparation of aqueous extract of *Fumaria vaillantii*, 25 gm powder of whole plant of *Fumaria vaillantii* was macerated with 150 ml of distilled water at 37 °C for 36 hrs with intermittent stirring. Later, the extract was filtered and filtered was dried followed by residue collection as powdered form.

In case of aqueous extract of *Benincasa hispida*, the juice of *Benincasa hispida* was dried by passing air over it using electric fan. When it became concentrated it was kept in an oven at 37 °C until it became paste form. This paste (residue) was used for the preparation of aqueous extract and it was prepared by dissolving 5 gm residue in 100 ml of distilled water and kept in an oven at 37 °C for 36 hrs with intermittent stirring. Later, the extract was filtered and was dried as before and obtained residue.

Chemicals

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

Animals

Young (2-month-old, 100 ± 2.23 g) and aged (14- to 16-month-old, 160 ± 10 g) male wistar rats were used. Six young and twelve aged male albino rats were selected for this experiment. The rats were acclimatized for a period of 15 days in our laboratory prior to the experiment. Animals were housed at an ambient temperature of 25 ± 2 °C with 12 hr light: 12 hr dark cycle. Animals were provided with animal food and water *ad libidum*.

Experimental design

Aged animals were divided into following two groups containing six rats in each group and young rats were used as younger control for the comparison with aged rat. Duration of the experiment was of 14 days. All the drugs were administered in oral rout by gavages.

Group I (Young control): Animals received only distilled water (0.5 ml / 100 g).

Group II (Aged control): Aged rats were received only distilled water (0.5 ml / 100 g) by gavages.

Group III (Extract treated aged group) Aged rats of this group supplemented with composite extract of whole plant of *Fumaria vaillantii* and ripe fruit extract of *Benincasa hispida* (1:1) at a dose of 2 mg/0.5 ml distille water/ 100 gm body weight/day for 14 days.

Pyloric ligation

After 14 days experiment, the animals of all groups including young control were fasted for 24 hrs. Under light ether anesthesia, the abdomen of each animal was opened through midline incision and the pyloric part was ligated (39). The stomach was placed in the usual position and then abdomen was sutured. Four hour later, pylorus ligated rats were sacrificed with anesththetic 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. Blood was collected from the dorsal aorta and serum was separated.

Measurement of pH of gastric secretion:

The pH of the gastric juice was measured by using pH meter.

Estimation of Free acidity and Total acidity:

The free acidity (free HCl) of the gastric juice was estimated by titration with N/10 NaOH solution, using Topfer's reagents (0.5 gm di ethyl amino azobenzenes /100 ml ethanol) as an indicator (40).

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 (40).

Pepsin concentration:

The pepsin in the gastric juice was estimated by the method of Samuual Natelson (41). 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 in gastric juice:

Vitamin-C level was measured using the 2, 4-dinitrophenyl hydrazine method (42, 43). This method measures the total vitamin-C concentration (ascorbic acid, dehydro ascorbic acid and di ketogulonic acid). 2 ml of 10% meta phosphoric acid was added to 0.5 ml of 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 thio urea and 10 µmol/L 2, 4 dinitrophenylhydrazine). The mixture was vortexed and stored in water bath at 37 °C for 3 hrs. The samples were then placed in ice for 10 minutes and were added to 2 ml of 12mol/L H₂SO₄ carefully. The absorbencies 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 hemoglobin percentage:

Hemoglobin percentage was determined by the cyanomethaemoglobin method (44).

Estimation of Serum Iron and Liver Iron:

Serum iron was determined by the method of International Committee for Standardisation in Hematology (45). Serum proteins were precipitated with a reagent containing trichloroacetic acid (TCA), hydrochloric acid and thioglycollic acid. After centrifugation supernatant was treated with bathophenanthroline reagent to give pink complex which was measured spectrophotometrically at 535 nm wave length.

Liver tissue was analyzed for nonheme iron by using the bathophenanthroline method as described by Torrance and Bothwell (46). The tissues were dried, weighed and digested with hydrochloric/trichloroacetic acids (TCA) at 65° C for 20 hr. The resulting mixture was reacted with bathophenanthroline reagent and absorbance was measured spectrophotometrically at 535 nm wave length.

Estimation of TBARS in Liver:

Lipid peroxidation was characterized by measuring the TBARS (47).

Histology of the stomach

The stomach of each animal was placed in Bouin's fluid for histological studies. Bouin's fixed stomach of each animal was processed for paraffin embedding. These paraffin blocks were then cut at 5 μ m thickness and stained with hematoxylin and eosin.

Statistical Analysis

Data were reported as means \pm SEM and one-way ANOVA followed by a multiple two-tail't' test was used for statistical analysis of the collected data. Differences were considered significantly at $P < 0.05$.

Results

The mean body weights of aged animals were markedly higher than those of young animals and weights of liver and kidney of aged control were significantly lowered in compared to young control group. Supplementation composite extract, a significant recovery was noted in organ weights in extract treated aged rats. There were no differences observed in food ingestion and water intake behavior throughout the experimental period.

Basal gastric secretion (Volume) was decreased significantly by 59.6 % in aged rat in comparison to young control group. Supplementation of aqueous extracts of the whole plant of *Fumaria vaillantii* and the ripe fruit of *Benincasa hispida* in combined to aged rat resulted an elevation in gastric secretion significantly by 24.39% in compare to aged control rats. The pH of the gastric juice was increased significantly by 52.12 % in aged control rats in comparison to young control. After supplementation of aqueous extract of the whole plant extract of *Fumaria vaillantii* and the fruit extract of *Benincasa hispida* in combined manner to aged rat, the pH of gastric juice was decreased significantly by 26.59 % in compare to aged control group. Supplementation of composite extract to the extract treated aged group resulted in recovery in pH of the gastric juice towards the young control group (Fig 1).

Both free acidity and total acidity were decreased significantly by 66.77 % and 16.97 % respectively in aged rat in compared to young control rats. After supplementation of composite extract to the extract treated aged group, a significant elevation in both the acidity were noted by 70.27 % and 9.46 % respectively in compared to aged control rats but free acidity was not reached to the young control level (Fig 1).

Pepsin concentration was decreased significantly by 11.07 % in aged rat in compared to young control. After supplementation of aqueous extract of the whole plant extract of *Fumaria vaillantii* and the fruit extract of *Benincasa hispida* in combined way to the aged rat, pepsin concentration was increased by 12.80 % in extract aged rats in compared to aged control (Fig2).

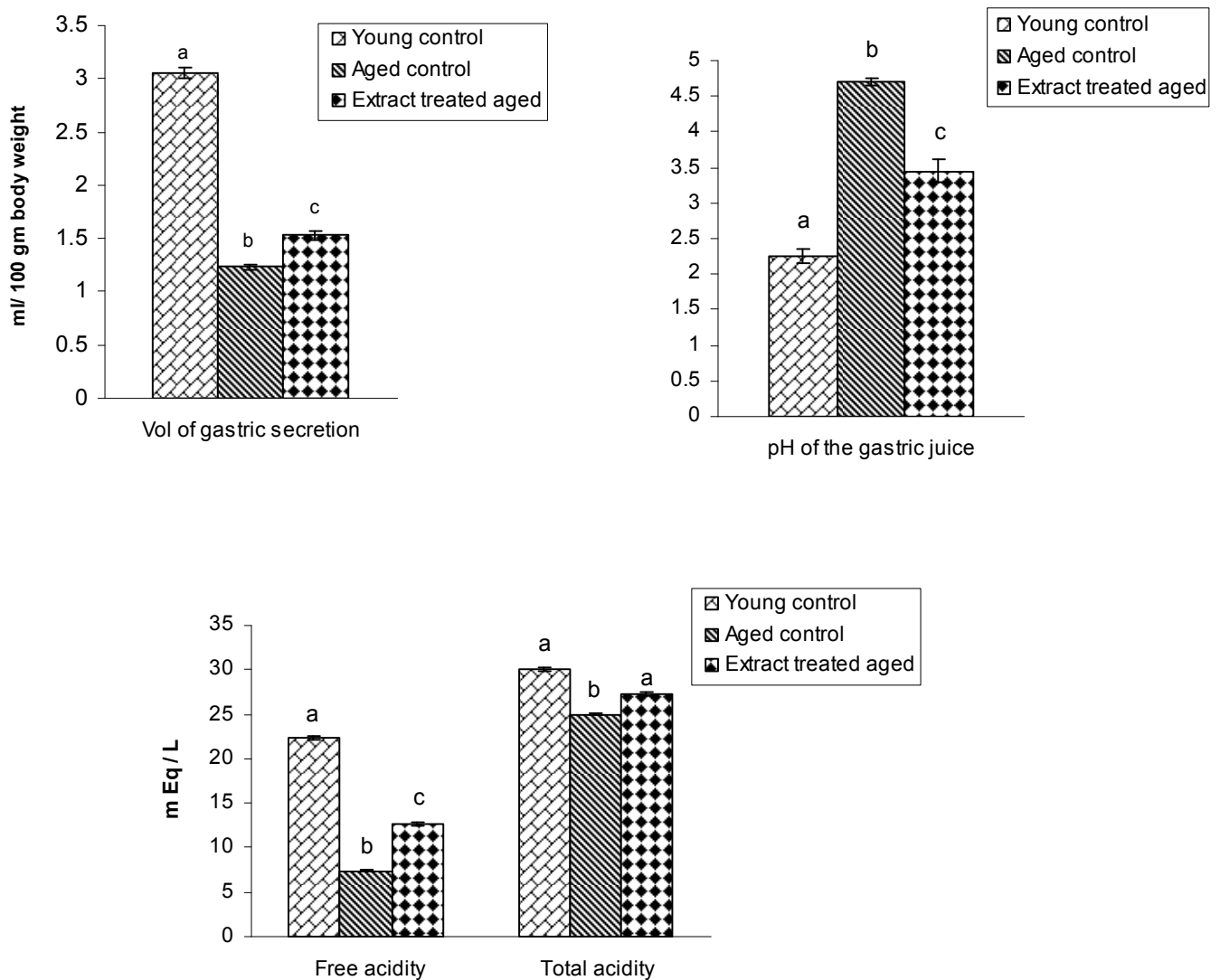


Fig 1: Corrective effect of aqueous extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* in a combined manner on volume of gastric secretion, pH of gastric juice, and on free acidity and total acidity in aged male albino rat. Data are expressed as Mean \pm SEM, n=6. Bars with different superscripts (a, b, c) differ from each other significantly (p<0.05). ANOVA followed by multiple comparisons two-tail “t” tests.

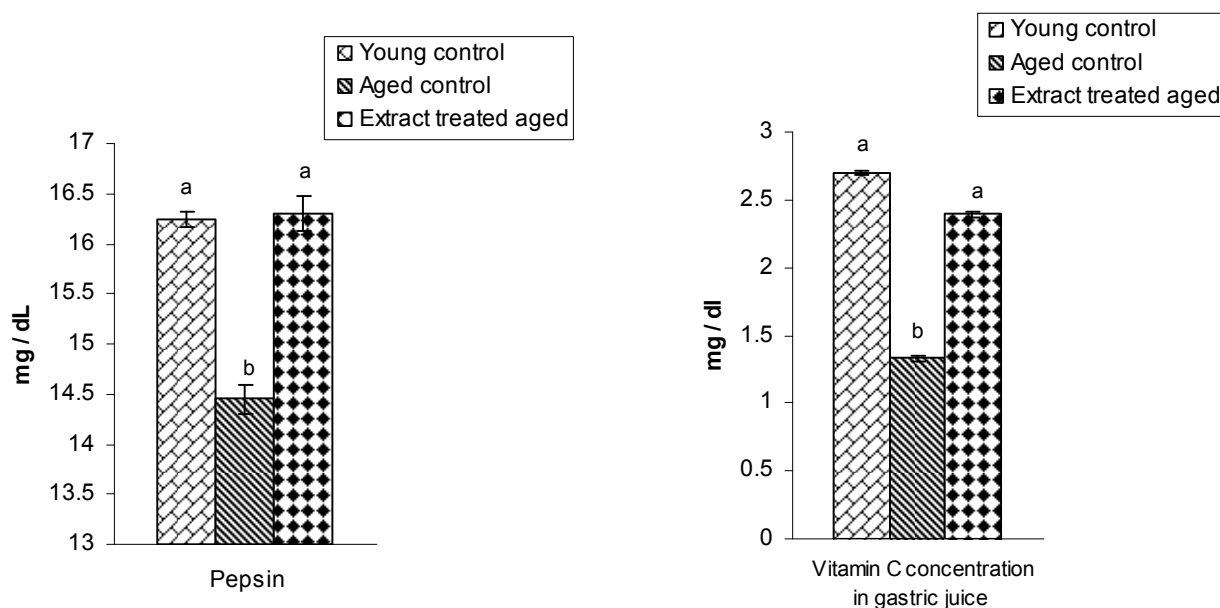


Fig 2 : Corrective effect of aqueous extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* in a combined manner on pepsin concentration and vitamin C concentration in gastric juice in aged male albino rat. Data are expressed as Mean \pm SEM, n=6. Bars with different superscripts (a, b, c) differ from each other significantly ($p < 0.05$). ANOVA followed by multiple comparisons two-tail “t” tests.

Table 1 Effect of aqueous extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* in a combined manner on body weight, hepato-somatic index and reno-somatic index in aged male albino rat.

Groups	Body Weight (gm)		Hepato-somaic index (gm/100 gm body weight)	Reno-somatic index (gm/100 gm body weight)
	Initial	Final		
Young Control	100.16 \pm 2.23 ^a	105.67 \pm 1.46 ^a	3.23 \pm 0.51 ^a	0.750 \pm 0.08 ^a
Aged Control	165.83 \pm 1.37 ^b	162 \pm 1.29 ^b	2.96 \pm 0.44 ^b	0.637 \pm 0.08 ^b
Extract treated aged	167.16 \pm 1.50 ^b	170.16 \pm 2.23 ^b	3.29 \pm 0.6 ^a	0.742 \pm 0.07 ^a

Data are expressed as Mean \pm SEM, n=6. Values with different superscript (a, b) in each vertical column differ from each other significantly ($p < 0.05$). ANOVA followed by multiple comparisons two-tail “t” test.

Vitamin C concentration in gastric juice was decreased significantly by 50.74 % in aged rat when compare to young control rats. After treatment of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida*, in combined manner, vitamin C in gastric juice was increased 79.69 % in extract treated aged rats compared to aged control and reached to the young control level (Fig 2).

The haemoglobin level of blood decreased significantly by 28.85 % in aged rat in compared to young control rats. After treatment of aqueous extract of the whole plant of

Fumaria vaillantii and the fruit of *Benincasa hispida*, in combined manner, hemoglobin level was increased significantly by 36.63 % in extract treated aged rats compared to aged control and reached to the young control level (Table 2).

Serum iron was decreased by 11.10 % and liver iron was increased by 95.18 % significantly in aged rat in compared to young control rats. After supplementation of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida*, in combined, serum iron was increased by 10.49 % in extract treated aged rats compared to aged control and reached to towards the young control level but liver iron decreased significantly by 41.65 % in extract treated group which fall down towards the young control level. (Table2).

Table 2 Effect of aqueous extract of whole plant of *Fumaria vaillantii* and ripe fruit of *Benincasa hispida* in a combined manner on the levels of haemoglobin, serum and hepatic iron, and TBARS in liver tissue in aged male albino rat.

Group	Hemoglobin (g/ dL)	Serum Iron (μ mol/ L)	Liver Iron (μ mol/ g dry wt)	TBARS in liver (n mol/ mg tissue)
Young Control	16.88 \pm 0.04 ^a	38.28 \pm 0.09 ^a	0.353 \pm 0.001 ^a	42.2 \pm 0.54 ^a
Aged Control	12.01 \pm 0.03 ^b	34.03 \pm 0.01 ^b	0.689 \pm 0.012 ^b	122.1 \pm 0.54 ^b
Extract treated aged	16.41 \pm 0.10 ^a	37.6 \pm 0.08 ^a	0.402 \pm 0.009 ^c	56.7 \pm 0.56 ^c

Data are expressed as Mean \pm SEM, n=6. Values with different superscript (a, b, c) differ from each other significantly ($p < 0.05$). ANOVA followed by multiple comparisons two-tail “t” test.

As shown in table 2, TBARS content i.e. lipid peroxidation in liver tissue was increased significantly by 189.3 % in aged rat when compare to young control rats. After treatment of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida*, in combined, TBARS content was reduced by 53.56 % in extract treated aged rats as compared to aged control group (Table2)

Histopathological examination of stomach mucosa shows the gastric mucosa focusing normal arrangement with normal glands in young control group (Plate 1a), endocrine cell hyperplasia and intestinal metaplasia in aged control group (Plate 1b) and Plate 1c shows recovery of gastric mucosa with appearance of normal gastric glands in extract treated aged group.

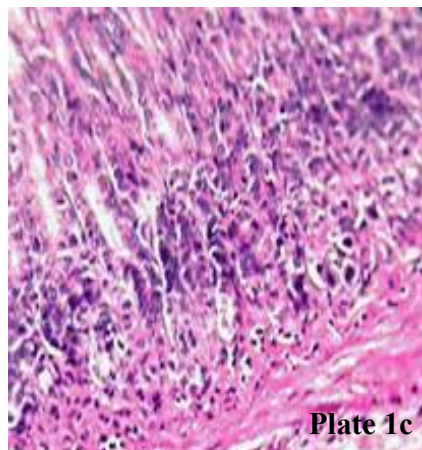
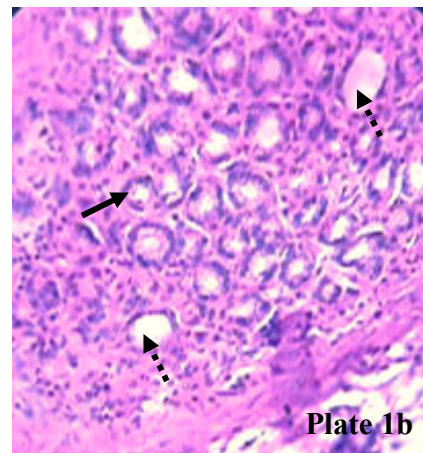
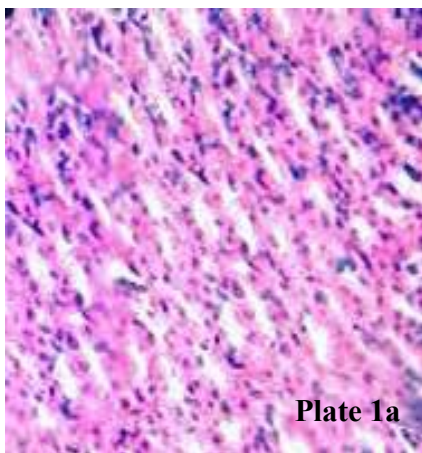


Plate 1: Representative photomicrographs of gastric mucosa focusing the arrangement with normal glands in young control group (Plate 1a), endocrine cell hyperplasia (indicated by →) and intestinal metaplasia (indicated by -.-→) in aged control group (Plate 1b) and Plate 1c shows recovery of gastric mucosa with appearance of normal gastric glands (Haematoxylin – Eosin staining , 400 X).

Discussion

In the present study the comparison was made of the gastric secretory activities between aged and young albino male rat. The another approach of this study is to find out the efficacy of the aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida* for the management of a aging induced hypochlorhydria in rat. Several studies have shown that the ability of gastric acid secretion is decreased with the advancement of age and it becomes half at the age over 60 years in respect to young (2,3). Gastric atrophy associated with aging and reduced gastric secretion. About 11% to 37% of the elderly have atrophic gastritis type B which results in elevated gastric pH (3). Our findings suggest that the volume of basal gastric secretion was reduced in aged control as compared to young control rat. After supplementation of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida* in composite manner to the aged rat, volume of the gastric secretion was increased significantly but not reached to young control group. We also noted the increased gastric pH of the aged control rat as compared with young rat. This low volume of gastric secretion with raised gastric pH may be the results of oxidative stress due to aging. The gastric acidity was decreased in aged rat in compared to young control rats as reported by others (48, 49). After supplementation of the composite extract, a significant increase was noted in both the free acidity and total acidity in compared to aged control rat but free acidity was not reached to the young control level. The possible explanations are that active ingredient (s) of composite extract may stimulate parietal cell renewal or preventing the gastric atrophy by stimulating the secretion of hydrochloric acid through acting on CNS as reported by others (50).

Low vitamin C concentration in gastric juice was noted here in aged rat with hypochlorhydria as reported by others (51). Intra gastric concentration of vitamin C was lowered in hypochlorhydric rat due to its instability at higher pH (52). Hypochlorhydria with low vitamin C concentration in gastric juice is a risk factor of gastric cancer (13) and also risk factor of *Helicobacter pylori* infection (14, 53). Gastric cancer rarely occurs before the age of 40 years, but its incidence increases subsequently with peak incidence occurring in the seventh decade of age of individual. Many probable reasons including altered carcinogen metabolism and long-term exposure of cancer-causing agents have been offered for the age-dependent rise in malignancies (12). After treatment of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida*, in composite manner to the aged group vitamin C level in gastric juice was increased in extract treated aged rat compare to aged control, and the level was reached to the reached to the young control level.

The haemoglobin level and serum iron were decreased significantly in aged rat in compare to young control rat but liver iron was high in aged control rats as compared to young rats. After treatment of aqueous extract of the whole plant of *Fumaria vaillantii* and the fruit of *Benincasa hispida*, in combined manner, hemoglobin level and serum iron were increased in extract treated aged rats compare to aged control and reached to the young control level. This recovery of the said parameter may be due to excess mobilization of iron from the liver and may stimulate haemoglobin synthesis.

Vitamin C also helps in iron absorption by reducing the ferric iron into soluble ferrous iron (54, 55). Non-haem iron requires an acidic pH for its absorption (56). In this present study, we found that vitamin C and gastric pH are not in favour of iron absorption in aged control group that lead to serum iron deficiency. Iron deficiency may reduce the hemoglobin biosynthesis and it leads to iron deficiency anemia (54, 57). It is not cleared that why liver loaded with extra iron in aged control rat. It has been revealed that aging is associated with increased lipid peroxidation and oxidative damage to tissues (25, 26, 29), and main causative agent is the extra iron in the liver which acts as a catalyst for lipid peroxidation (31). In this present study, we found that TBARS was increased in aged control group with elevated liver iron in compared to young control. Elevated TBARS reflects an increased lipid peroxidation in the liver in aged rats as supported by others (32). Supplementation of this composite extract reduced the lipid peroxidation induced by over load of iron in the liver due to aging. The composite extract may induce the mobilization of iron from the storage organ like liver. Thus this extract supplementation increased the serum iron along with percentage of haemoglobin in extract treated aged rat as compared to aged control. So this present study suggests that the composite extract of above mentioned plants may have antioxidant property in preventing these changes.

Histopathological study of the stomach revealed that endocrine cell hyperplasia and intestinal metaplasia were noted in aged control group as reported by others (58) but it is interesting that after supplementation of composite extract in the extract treated aged group, the mucosa was found to be almost normal with a few endocrine cells which comparable to young control rat. So, it may be concluded that the composite extract of above mentioned plants has the beneficial role on stomach of aged rats in prevention of gastric atrophy and metaplasia. Beside these it has also anti lipid peroxidative activity.

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