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Full length Research Article

Age-Related Effects of Carbohydrate-, Protein- And Fat-Rich Diets on Healing of Acetic Acid-Induced Gastric Ulcers in Rats.

Akpamu U.[1,2], Salami A.T.[1], Odukanmi O.A.[1], *Olaleye S.B.[1]

[1]Laboratory for Gastrointestinal Secretion and Inflammatory Research, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
[2]Department of Physiology, Federal University Oye-Ekiti, Ekiti, Nigeria.

Summary: In this study, we compared the ulcer healing effect of carbohydrate-, protein- and fat-rich diets on acetic acid-induced ulcers in young and aged rats. Male Wistar rats (40 each at 3-, 6-, 12-, and 18 months old) were grouped into four to receive basal diet (control), carbohydrate-, protein-, or fat-rich diets for 21 days before acetic acid-induced gastric injury. After this, the various feedings continued for 3- and 7 days. Planimetry was used for the ulcer healing study. We estimated the redox status, pepsin, mucin, and nitric oxide activities by UV/Vis-spectrophotometer while the Epidermal Growth Factor-Receptor (EGF-R) was by immunohistochemistry. Data was analyzed (two-way ANOVA) and was considered significant at $p \leq 0.05$. Percentage ulcer healing by day 7 relative to day 3 decreased with advancing age in other diets but increased in the aged rats fed a protein-rich diet. Gastric carbonyl, malondialdehyde, and pepsin activities increased significantly with age, while superoxide dismutase, catalase, mucin, Nitric-oxide and EGF-R expression significantly decreased with age. Protein-rich diets modulated the age-related alterations. These findings suggest that a protein-rich diet facilitates the healing of acetic acid-induced gastric injury by enhancing gastroprotective activity to favour EGF-R expression in the ulcerated stomach.

Keywords*: Ageing, ulcer healing, protein, carbohydrate, fat, rats*

**Authors for correspondence: sbolaleye@yahoo.com, Tel: +2348023255893*

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INTRODUCTION

The percentage of the population over 65 years old is rising and is expected to increase to 21% by the year 2040 in the United States, with an increase in age-related diseases (Alan, 2017). During ageing there are structural and functional changes in multiple organs with associated increased inflammation (Guarner and Rubio-Ruiz, 2015) in the skin, lungs, digestive system and kidneys (Goldacre, 2009; Alan, 2017). Among these systems, the digestive system is the primary system that has the largest contact area with ingested pathogens and environmental factors (Soenen *et al*., 2016), which incessantly exposes gastric mucosa to the action of various irritants capable of injuring its structural and functional integrity to cause ulceration.

Gastric ulceration is a disease of chronic development characterized by necrotic abrasion, laceration, or injury in the mucosal and muscularis mucosae layer of the digestive tract. Considering its morbidity and mortality rates, digestive tract ulcer is among the most prevalent diseases affecting people worldwide (Xie *et al*., 2022). The leading cause is not well-known, but an imbalance between aggressive and cytoprotective factors in the mucosa is broadly recognized (Zhang *et al*., 2020). The multifactor-associated etiology includes Helicobacter pylori infection, genetic, free radicals formation, prolonged and excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, cigarette smoking, and stress (Akpamu *et al*., 2016). Others may include nutritional deficiencies and poor dietary habits (Yegen, 2018). These conventional risk factors cannot explain several digestive tract ulcers, thereby supporting the possibility of other factors.

Reports show that digestive tract ulceration and its associated disorders and dysfunctions increase with ageing (Polo *et al*., 2012) due to changes in the defense and repair processes (Campos *et al*., 2000). The healing of ulcerated gastric mucosa, assessed by area and depth in rats, has been reported to be facilitated in young rats compared to aged rats (Ajayi and Olaleye, 2015). The ulcerated digestive tract healing is a dynamic process involving alleviating aggressive factors, deposition of epithelial and connective tissue cells, and mucosal reconstruction (Tarnawsaki *et al*., 2001). These processes are controlled by interacting with factors like diet, feeding habits, and pattern (Ogias *et al*., 2010) and growth factors (Osaki *et al*., 2011). Previous studies have reported epidermal growth factor receptor (EGF-R) as one of the ideal growth factors for wound healing, which can mitigate ulceration through its mechanism in the gastrointestinal tract (Khanbanha *et al*., 2014).

There are existing facts that ageing correlates positively with deceased mucosa protective factors (Polo *et al*., 2012) and ulcer healing (Ajayi and Olaleye, 2015). Therefore, there is concern about this population's digestive tract health status. Considering that reports on the investigation of the interaction between substances ingested, such as diet, their influence on gastric ulcer healing and gastric protective factors is unavailable in different ages. The main objective of the present study is to compare the effect of the carbohydrate-, protein- and fat-rich diet on the healing process of acetic acid-induced ulcers in young and aged rats.

MATERIALS AND METHODS

Animals and ethical approval of the study: Forty male Wistar rats were assigned and used per phase in this study at 3-, 6-, 12-, and 18 months old. To ascertain the ages of the rats, they were bred from birth in the Postgraduate Animal House, Department of Physiology, University of Ibadan, Ibadan, Nigeria, from parents obtained at the Central Animal House, College of Medicine, of the same institution. The housing of the rats was clean wire meshed plastic cages in a day-light and night-dark cycle. The rats were fed with designated feeds and allowed drinking water ad libitum.

Ethical approval was sought and obtained for the study from the Animal Care and Use Research Ethics Committee of the University of Ibadan with assigned number UI-ACUREC/18/0100. The investigation was conducted strictly following the approved guidelines and regulations of UI-ACUREC and in compliance with the International Guidelines for Laboratory Animal Care and Use Act of the National Institute of Health (NIH, 1985).

Experimental design: Rats were randomly grouped into four diet schemes for four age groups (Figure 1) as follows;

- Group 1: Control (10 rats each at 3-, 6-, 12- and 18-months); rats fed formulated standard diet (basal diet).
- Group 2: Carbohydrate group (10 rats each at 3-, 6-, 12- and 18-months); rats fed on a carbohydrate-rich diet.
- Group 3: Protein group (10 rats each at 3-, 6-, 12- and 18 months); rats fed on a protein-rich diet.
- Group 4: Fat group (10 rats each at 3-, 6-, 12- and 18 months); rats fed a fat-rich diet.

The diet formulation were based on different macro-nutrients, as described in a previous study by Adedeji and Olapade-Olaopa (2018). Rats were fed on these diets for 21 days before ulcer induction and continued for 3 and 7 days, during which we harvested five rats' stomachs for ulcer healing study.

Induction of acetic-acid ulcer model: The acetic acid ulcer model was used in this study because the model easily and reliably produces round, deep ulcers and high resemble human ulcers in both pathological features and healing processes (Okabe and Amagase, 2005). Acetic acid ulcer was induced as described by Ajayi and Olaleye (2015) with some modifications. Laparotomy was performed under a cocktail of anaesthesia (Ketamine, 0.0015ml/100g bwt and Xylazine, 0.0005ml/100g bwt). The stomach was exposed and gastric ulcer was induced by clamping the anterior and posterior glandular stomach walls with 9mm eye forceps, and 0.2 mL of 30% acetic acid solution was slowly injected into the secured area for 60 seconds and withdrawn. After rinsing the abdomen with normal saline, the abdominal openings were sutured back. Appropriate topical antibiotics were applied for 3 days on the surgical site to prevent infection. The rats were weighed every other day to determine their body weight, and the ulcerated stomachs were harvested 3- and 7 days post-ulceration, weighed, and harvested for macroscopic scoring and biochemical assays.

Asseement of the gross gastric ulcer scores: The stomachs were excised and cleansed using a phosphate buffer and placed it on filter paper to dry. The vertical and horizontal diameters of the formed gastric ulcer were measured using a plastic ruler under a magnifying lens. Ulcer Indexes (UI), Ulcer Area (UA), and Ulcer Healing Rate (UHR) were determined as described by Xiaoyum *et al*. (2007) with some modifications. The sum of the longest vertical and horizontal diameters across the formed ulcer was calculated as UI.

UA (mm^2) = π x (sum of vertical diameters / 2) x (sum of transverse diameters / 2).

Percentage ulcer healing (%H) was calculated by day 7 relative to day 3 as:

%H = ((UI at day 3 - UI at day 7) / (UI at day 3)) x 100%.

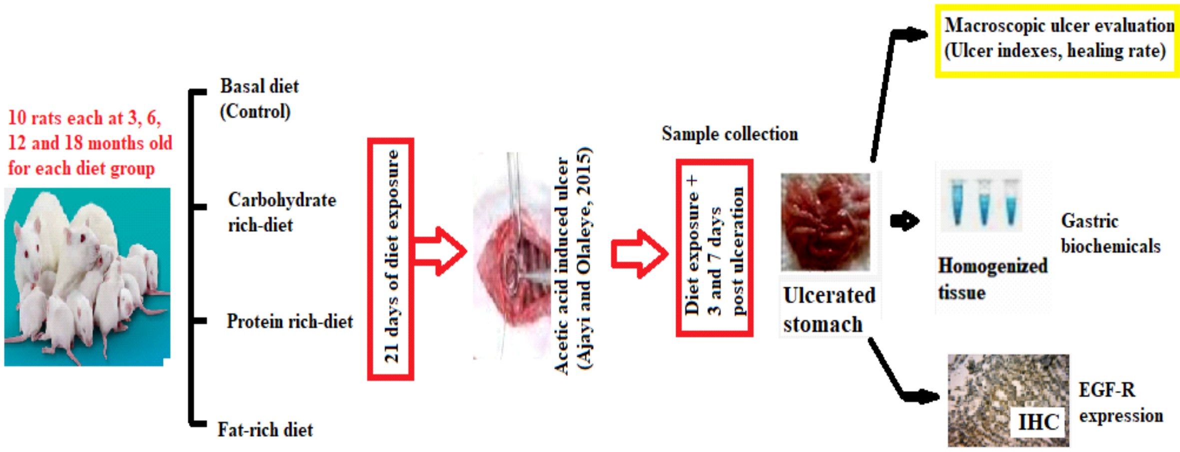


Figure 1:
Experimental design

Gastric biochemicals analysis: The cleansed harvested ulcerated stomachs were minced in ice-cold 0.1M phosphate buffer solution (10% of volume) and centrifuged (via a cold centrifuge) at 4°C (5,000 rpm for 15 minutes) as previously described (Salami *et al*., 2021). The supernatants were used for the estimations of gastric biochemical assays. Gastric tissue protein was estimated using the Biuret method. Malondialdehyde (MDA) levels were assessed by evaluating thiobarbituric acid reactive substances (TBARS) and mucin was estimated based on hexose component determination (Salami *et al*., 2021). The gastric carbonyl, catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), nitric oxide (NO) and pepsin activities were according to standardized method in Salami *et al*. (2021).

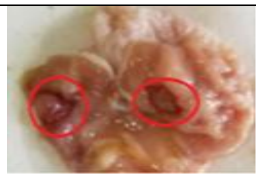
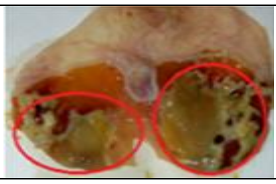
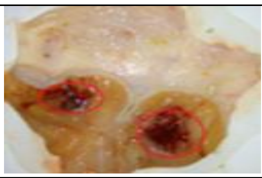
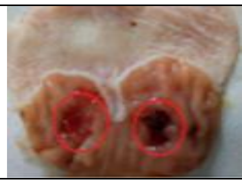
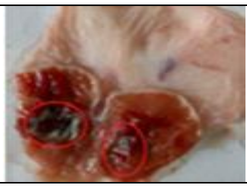
Immunohistochemistry study: Immunohistochemical staining was performed on the ulcerated stomach (5µm thickness of fixed stomach tissues embedded in paraffin) using EGF-R (Catalog No.: E-AB-31285) monoclonal antibodies in immunoperoxidase techniques as modified by Oyagbemi *et al*., (2018). The prepared slides were viewed and photomicrographs were taken at x40, x100 and x400 magnification with a digital microscope (VJ-2005 DN MODEL BIO-MICROSCOPR(R) and the immunoreactivity expression was quantified using TS View XC Image (R) Software, File version 6.2.5.3 and ImageJ (version 1.51).

Statistical analysis: Data was analyzed and presented as mean ± SEM of 5 rats in a group. Using GraphPad Prism (Version 5.0), two-way variant analysis and the Bonferroni post hoc test were considered significant at $p \leq 0.05$.

RESULTS

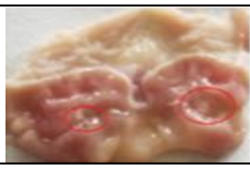
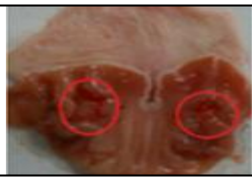
Effect of carbohydrate-, protein- and fat-rich diets on relative stomach weight, gross ulcer area, and percentage ulcer healing in rats of different ages with acetic acid-induced gastric ulcer: The relative stomach weight decreases with ageing; however, there is no significant difference in the relative weight of the stomach between or within groups (Tables 1 and 2). The mean gross ulcer area of the control, carbohydrate- and fat-rich diet groups increased significantly with age. In contrast, that of the protein-rich diet decreased with age and was significantly lower than the control. On the other hand, the percentage of ulcer healing by day 7 relative to day 3 was increased considerably in the aged rats fed a protein-rich diet compared to the control.

Table 1:
Effect of carbohydrate-, protein- and fat-rich diets on relative stomach weight, gross ulcer area and percentage healing by day 3 of acetic acid ulcer healing in rats of different ages

Age	Standard diet	CHO diet	PRO- diet	FAT diet
3 months				
RSW	1.09±0.07	0.87±0.00	1.12±0.09	0.91±0.08
GUA	12.83±0.66	5.76±2.92*	5.24±4.12*	7.86±2.98
6 months				
RSW	0.93±0.11	0.78±0.02	1.09±0.09	0.87±0.08
GUA	24.88±0.94^{a}	2.10±0.52*	3.67±1.71*	4.98±0.69*
12 months				
RSW	1.13±0.04	0.89±0.04	1.10±0.04	1.04±0.16
GUA	43.74±1.12^{ab}	26.98±2.23^{ab*}	32.48±0.79^{ab*}	25.67±0.43^{ab*}
18 months				
RSW	1.11±0.11^{a}	0.93±0.08	0.92±0.10	1.06±0.13
GUA	46.10±1.32^{ab}	42.43±1.08^{abc}	28.29±1.69^{ab*}	36.67±1.22^{ab*}

The values are shown as mean ± SEM; α= 0.05; n = 5.
** versus age-matched basal diet, a versus 3 months, b versus 6 months, c versus 12 months. Basal = Standard diet, CHO = Carbohydrate rich-diet, PRO^{-} = Protein rich-diet, FAT = Fat rich-diet, RSW = Relative Stomach Weight, GUA = Gross Ulcer Area.*

Table 2:
Effect of carbohydrate-, protein- and fat-rich diets on relative stomach weight, gross ulcer area and percentage healing by day 7 of acetic acid ulcer healing in rats of different ages

Age	Standard diet	CHO diet	PRO- diet	FAT diet
3 months				
RSW	1.21±0.05	0.80±0.03	1.08±0.11	1.16±0.07
GUA	6.29±6.29	0.00±0.00	8.38±4.19	0.00±0.00
%H	88.89±1.11	100.00±0.00	69.94±3.79*	100.00±0.00
6 months				
RSW	1.04±0.09	0.71±0.05	1.08±0.05	1.09±0.08
GUA	13.62±1.05	9.43±3.14	13.62±2.10	20.95±2.02[a]
%H	85.11±3.03	68.33±4.4[a*]	77.78±11.11	56.94±1.95[a*]
12 months				
RSW	0.96±0.09	0.72±0.04	0.97±0.03	0.91±0.10
GUA	53.43±3.65[ab]	49.24±3.49[ab]	4.19±2.10*	60.10±1.56[ab]
%H	63.03±2.13[ab]	55.99±2.18[a]	96.68±1.68*	54.23±1.65[a]
18 months				
RSW	0.80±0.04	0.75±0.04	0.93±0.05	0.83±0.04
GUA	74.38±2.09[abc]	68.10±4.88[abc]	0.00±0.00[a*]	62.86±1.43[ab*]
%H	57.82±5.27[ab]	54.86±5.62[a]	100.00±0.00[a*]	61.16±0.67[a]

Values are expressed as mean ± SEM; α= 0.05; n = 5. versus age-matched Standard diet, a versus 3 months, b versus 6 months, c versus 12 months*
***Keys:** Basal = Standard diet, CHO = Carbohydrate rich-diet, PRO- = Protein rich-diet, FAT = Fat rich-diet, RSW = Relative Stomach Weight, GUA = Gross Ulcer Area, %H = percentage Ulcer Healing.*

Effect of carbohydrate-, protein- and fat-rich diets on gastric redox level in rats of different ages with acetic acid-induced gastric ulcer: Gastric carbonyl and MDA levels increased significantly with age in all the treated groups. However, the gastric MDA level increased considerably in the aged rats fed carbohydrate- and fat-rich diets compared with the control. Gastric SOD and CAT decreased substantially with age, while gastric GSH was insignificant with age. The different fed diets significantly increased gastric SOD levels in the 18 months old compared to the age-match control by day 7 of ulcer healing. In contrast, CAT levels increased significantly in the 18-month-old fed fat-rich diet and the 3-month-old of the different diets compared with the control (Tables 3 and 4).

Effect of carbohydrate-, protein- and fat-rich diets on gastric pepsin, mucin, and nitric oxide levels in rats of different ages with acetic acid-induced gastric ulcer: Gastric pepsin level increased significantly with age by days 3 and 7 of ulcer healing but was insignificant between the other diets compared with the control by day 7; however, it increased significantly in the 6- and 18-months fed protein- and fat-rich diet and in the 6-months old carbohydrate-rich diet compared with the control by day 3, (Tables 5 and 6). On the other hand, gastric mucin and nitric oxide levels decreased significantly with age by days 3 and 7 of ulcer healing. Gastric mucin levels increased significantly in the 12-nth-old fed a protein-rich diet, while nitric oxide was lower in the 18-month-old compared with the control by day 7 of ulcer healing. Also, carbohydrate- and fat-rich diets significantly increased nitric oxide levels compared with the control by day 7 of ulcer healing.

Effect of carbohydrate-, protein- and fat-rich diets on gastric expression of EGF-R in rats of different ages with acetic acid-induced gastric ulcer: Gastric expression of EGF-R decreased with ageing in all the groups and was significantly lower by day 3 of ulcer healing in the 12-month-old protein- and fat-rich and 18-month-old of fat-rich diets compared with the control. By day 7 of ulcer healing, gastric expression of EGF-R was 83.76±1.73%, 74.30±2.31%, 72.39±1.20%, and 71.99±0.58% for 3-, 6-, 12-, and 18-months old respectively for the control (Figure 2). Gastric expressions of EGF-R for the protein-rich diet-fed rats were 87.42±1.27%, 87.34±4.06%, 83.26±2.33%, and 74.01±2.31% for the respective ages, while it decreased for carbohydrate- (75.49±3.96%, 69.14±1.63%, 68.21±1.92%, and 63.49±1.75%) and fat-rich diets (77.64±2.88%, 76.71±3.54%, 74.55±2.28%, and 63.62±1.84%) compared with the control (Figure 2).

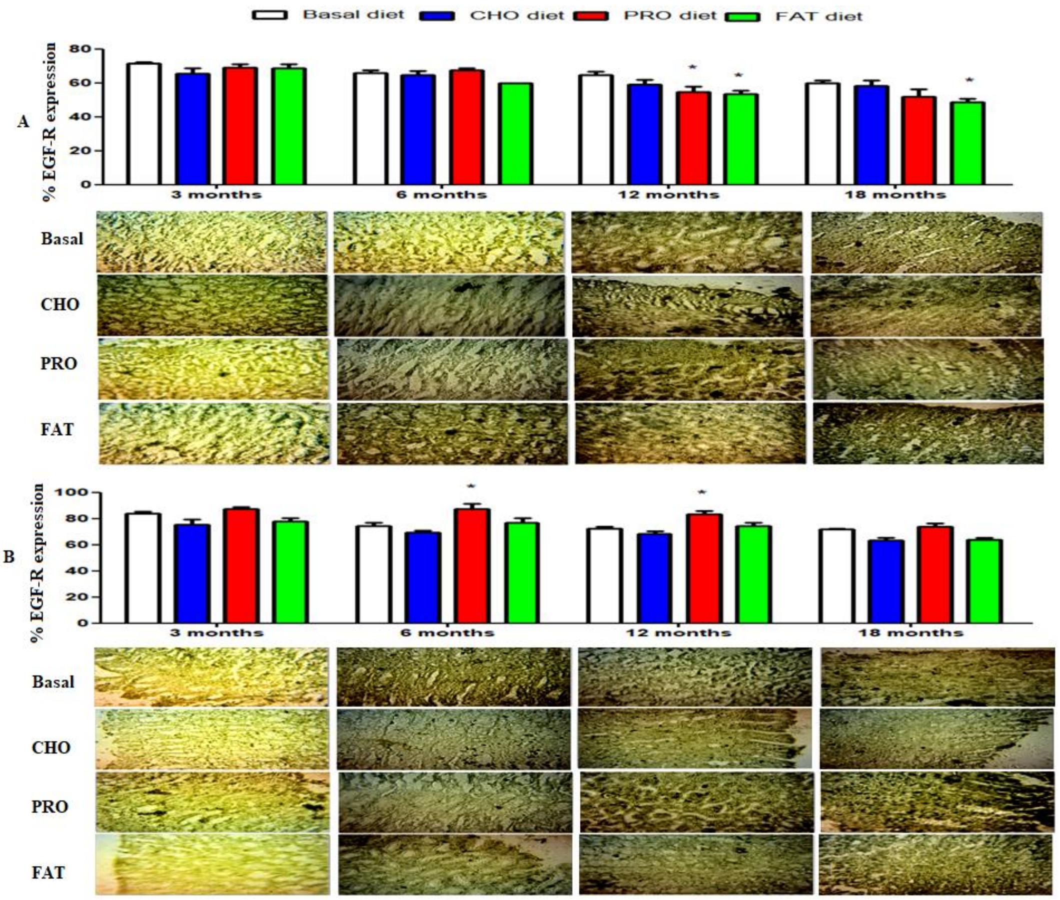


Figure 2:
Effect of carbohydrate-, protein- and fat-rich diets on gastric expression of EGF-R by day 3 (a) and 7 (b) in rat of different ages
Values are expressed as Mean ± SEM; n = 5. significant versus age-matched control diet, Basal = Control diet, CHO = Carbohydrate rich-diet, PRO- = Protein rich-diet, FAT = Fat rich-diet.*

Table 3:
Effect of carbohydrate-, protein- and fat-rich diets on gastric redox level by day 3 of acetic acid ulcer healing in rats of different ages

	Age	Basal	CHO	PRO-	FAT
Carbonyl	**3 months**	1.54±0.03	1.28±0.05	1.53±0.02	1.56±0.02
(x 10^{-7} ng mL^{-1})	**6months**	1.61±0.11	1.41±0.01	1.51±0.02	1.75±0.09
	12 months	1.73±0.06	1.64±0.02	1.58±0.02	1.85±0.06
	18 months	1.85±0.05	1.97±0.03[a]	1.77±0.06	2.05±0.02[a]
MDA	**3 months**	3.50±0.24	3.20±0.29	3.68±0.71	3.37±0.48
(x 10^{-6} ng mL^{-1} Protein)	**6months**	4.11±0.46	3.62±0.71	4.48±0.56	3.60±0.17
	12 months	5.41±0.44	5.41±0.79	5.36±0.72	7.06±0.47[ab]
	18 months	5.94±0.53[a]	5.99±0.84	6.43±0.45	6.98±0.16[ab]
SOD	**3 months**	11.34±1.26	11.15±0.33	11.55±0.81	10.94±0.16
(ng mL^{-1} protein)	**6months**	10.50±0.62	9.68±0.44	10.76±0.17	9.41±0.27
	12 months	10.47±0.60	9.41±0.89	9.74±0.76	7.83±0.25[a*]
	18 months	9.49±0.75	8.23±0.91	9.52±0.71	8.56±0.84[a]
CAT	**3 months**	6.98±0.20	7.60±0.09	7.98±0.26[*]	7.75±0.20
(x 10^{2} ng mL^{-1} Protein)	**6months**	6.60±0.39	6.65±0.48[a]	6.11±0.22[a]	6.33±0.28[a]
	12 months	4.86±0.27[ab]	5.45±0.16[ab]	4.33±0.22[ab]	4.25±0.14[ab]
	18 months	4.26±0.36[ab]	3.66±0.01[abc]	3.13±0.15[abc]	2.69±0.30[abc*]
GSH	**3 months**	3.79±0.05	3.66±0.03	3.68±0.04	3.72±0.05
(mg dL^{-1})	**6months**	3.65±0.05	3.82±0.01	[a]3.88±0.02	3.98±0.12
	12 months	3.72±0.07	4.01±0.14	3.83±0.06	3.82±0.05
	18 months	3.77±0.06	3.81±0.16	[a]3.94±0.01	3.79±0.13

Values are expressed as Mean ± SEM; n = 5. significant versus age-matched control diet, a versus 3 months, b versus 6 months, c versus 12 months. Basal = Control diet, CHO = Carbohydrate rich-diet, PRO- = Protein rich-diet, FAT = Fat rich-diet*

Table 4:
Effect of carbohydrate-, protein- and fat-rich diets on gastric redox level by day 7 of acetic acid ulcer healing in rats of different ages

	Age	Basal	CHO	PRO-	FAT
Carbonyl ($\times 10^{-7}$ ng mL^{-1})	**3 months**	1.48±0.07	1.36±0.01	1.42±0.09	1.42±0.01
	6months	1.58±0.05	1.63±0.08	1.52±0.01^{a}	1.60±0.01
	12 months	1.79±0.02	1.72±0.07	1.93±0.01	2.15±0.09^{a}
	18 months	2.19±0.08^{ab}	2.44±0.01^{abc}	2.17±0.05^{ab}	2.51±0.02^{ab}
MDA ($\times 10^{-6}$ ng mL^{-1} Protein)	**3 months**	3.08±0.45	4.27±0.43	4.05±0.17	4.21±0.21
	6months	3.96±0.80	4.43±0.44	4.33±0.42	5.52±0.40
	12 months	4.19±0.23	5.92±0.15*	5.50±0.31	7.72±0.60^{a*}
	18 months	4.58±0.36	6.54±0.31^{ab*}	5.73±0.55	8.16±0.88^{a*}
SOD (ng mL^{-1} protein)	**3 months**	10.14±0.53	10.61±0.86	11.51±0.21	12.94±1.03*
	6months	9.01±0.50	10.74±0.50	10.93±0.02*	10.39±0.27
	12 months	8.77±0.04	8.86±0.49	9.78±0.55	10.32±0.25
	18 months	7.08±0.45^{a}	8.98±0.48*	9.46±0.42^{a*}	9.71±0.10^{a*}
CAT ($\times 10^{2}$ ng mL^{-1} Protein)	**3 months**	5.34±0.47	6.32±0.22*	6.34±0.16*	6.65±0.14*
	6months	5.00±0.23	4.55±0.27^{a}	4.73±0.39^{a}	5.38±0.21^{a}
	12 months	4.45±0.13	4.30±0.17^{a}	4.50±0.19^{a}	4.38±0.25^{ab}
	18 months	2.06±0.21^{a}	1.88±0.26^{abc}	2.12±0.42^{abc}	3.11±0.07^{abc*}
GSH (mg dL^{-1})	**3 months**	4.05±0.43	3.78±0.04	3.73±0.05	3.85±0.09
	6months	4.04±0.01	3.84±0.05	3.78±0.10	3.75±0.05
	12 months	3.80±0.07	4.06±0.11	3.78±0.02	3.79±0.07
	18 months	3.72±0.04	3.76±0.06	3.30±0.09^{a}	3.67±0.06

Values are expressed as Mean ± SEM; n = 5. significant versus age-matched control diet, a versus 3 months, b versus 6 months, c versus 12 months. Basal = Control diet, CHO = Carbohydrate rich-diet, PRO^{-} = Protein rich-diet, FAT = Fat rich-diet*

Table 5:
Effect of carbohydrate-, protein- and fat-rich diets on gastric pepsin, mucin and nitric oxide levels by day 3 of acetic acid ulcer healing in rats of different ages

Gastric parameter	Age	Basal	CHO	PRO-	FAT
Pepsin ($\times 10^{2}$µg mL^{-1})	**3 months**	11.62±0.21	12.49±0.32	12.19±0.69	10.39±0.28
	6months	11.89±0.15	13.81±0.36*	13.20±0.80*	13.88±0.65^{a*}
	12 months	12.85±0.41	14.20±0.09	13.90±0.27	13.74±0.35^{a}
	18 months	13.71±0.24	13.49±0.57	15.35±0.45^{a*}	15.41±0.23^{a*}
Mucin (mg dL^{-1})	**3 months**	56.64±6.71	48.81±4.08	39.34±1.10*	45.89±0.92
	6months	45.70±0.88	40.24±2.03	43.03±4.10	47.51±3.42
	12 months	51.42±5.52	37.04±2.84*	46.43±1.37	31.35±4.80*
	18 months	35.08±4.40	31.94±4.09^{a}	27.48±2.82^{abc}	32.53±3.13
Nitric oxide (mL organ weight^{-1})	**3 months**	40.43±1.15	43.31±1.34	43.93±1.30	50.96±1.41*
	6months	43.85±4.29	43.97±2.78	42.24±2.33	47.39±1.02
	12 months	38.06±4.55	42.02±1.95	40.72±3.62	41.13±3.41
	18 months	29.11±4.40	32.89±2.17^{a}	35.86±1.86	27.62±1.40^{abc}

Values are expressed as Mean ± SEM; n = 5.* significant versus age-matched control diet, a versus 3 months, b versus 6 months, c versus 12 months. Basal = Control diet, CHO = Carbohydrate rich-diet, PRO^{-} = Protein rich-diet, FAT = Fat rich-diet

Table 6:
Effect of carbohydrate-, protein- and fat-rich diets on gastric pepsin, mucin and nitric oxide levels by day 7 of acetic acid ulcer healing in rats of different ages

Gastric parameter	Age	Basal	CHO	PRO-	FAT
Pepsin ($\times 10^{2}$ µg mL^{-1})	**3 months**	11.31±0.66	12.54±0.30	11.24±0.30	12.17±0.30
	6months	12.31±0.22	13.07±0.19	11.81±0.43	13.31±0.55
	12 months	13.10±0.17^{a}	14.02±0.23^{a}	12.18±0.60	13.50±0.49
	18 months	14.05±0.10^{a}	14.24±0.14^{ab}	12.97±0.34	13.80±0.16
Mucin (mg dL^{-1})	**3 months**	52.51±1.82	57.35±3.53	61.72±1.86	52.21±0.86
	6months	49.10±4.80	45.52±4.89	54.33±2.56	46.39±2.36
	12 months	32.69±1.35^{ab}	37.63±2.62^{a}	43.40±3.31^{a*}	38.24±0.59^{ab}
	18 months	28.67±2.71^{ab}	28.22±2.57^{a}	35.83±1.56^{ab}	27.62±0.85^{abc}
Nitric oxide (mL organ weight^{-1})	**3 months**	57.53±7.24	69.19±2.75*	59.50±3.44	58.42±1.08
	6months	49.86±1.54	58.94±2.02*	46.91±3.28	52.68±1.56*
	12 months	45.24±3.19	49.98±1.02^{ab}	34.78±2.29^{a}	35.10±1.18^{ab}
	18 months	43.17±2.78	31.07±0.21^{abc*}	27.17±4.78^{ab*}	30.66±0.65^{ab*}

Values are expressed as Mean ± SEM; n = 5.* significant versus age-matched control diet, a versus 3 months, b versus 6 months, c versus 12 months. Basal = Control diet, CHO = Carbohydrate rich-diet, PRO^{-} = Protein rich-diet, FAT = Fat rich-diet.

DISCUSSION

Gastrointestinal ulcer is already known to have a multifactor-associated etiology and in the present study, we investigated the fate of diets rich in carbohydrate, protein and fat on the ulcer healing in young and aged rats. The findings agreed with the previous fact that ulcer healing is delayed in the old rats compared to the young rats if one considers the healing rate in the control, carbohydrate- and fat-rich diets but not so for the protein-rich diet. A protein-rich diet enhances healing in the aged rats and thus suggests that a protein-rich diet may favour ulcer healing for older adults. Based on results in the production and prevention of experimental gastric ulcer in animals, Windwer and Matzner (1938) has previously reported and suggested a high-protein diet to relieve ulcer symptoms in 90% of studied patients. The report concurs with the present findings.

Considering the multifactor aetiology of digestive tract ulceration, including free radicals and oxidative formation, studying the gastric redox system is necessary. In the present study, ageing favours excess ROS over endogenous antioxidants, which may have delayed ulcer healing in the aged rats. However, a protein-rich diet inhibits the gastric concentration of protein carbonyl and MDA as well as stimulates SOD to favour ulcer healing as compared to carbohydrate-and fat-rich diets that promote protein carbonyl and MDA and inhibit the endogenous antioxidants. In line with this finding, high carbohydrate and fat diets generate increased levels of lipid peroxidation and protein carbonylation products and inhibit endogenous antioxidants (Apel and Hirt, 2004).

Pepsin has a mucolytic activity and digests the adherent luminal mucus layer. This may explain the carbohydrate- and fat-rich diets-induced inhibition of mucin levels since they stimulate pepsin activity in the ulcerated stomach during gastric ulcer healing. However, ageing is associated with a reduced capacity of many mucus-secreting cells (Newton et al., 2003). Peptic ulcer diet therapy aims to prevent the hypersecretion of peptic chloride and promote healing based on a complex sequence of events going from the initial trauma to the repair of the damaged tissue. In this study, carbohydrate- and fat-rich diets stimulate gastric pepsin and nitric oxide levels during the healing phase of acetic acid-induced gastric ulcers, while protein-rich diets boost mucin levels. This mucin effect of a protein-rich diet may have protected the gastric mucosa from acetic acid-induced damage or enhanced repair of the ulcerated mucosa.

The findings of the present study suggest that a protein-rich diet up-regulated gastric EGF-R in ulcerated rats. EGF binds to its receptor, EGF-R, to halt acid secretion, exerts a trophic effect on gastroduodenal mucosa, protects gastric mucosa against injury, and accelerates gastroduodenal ulcer healing by stimulating cell migration and proliferation (Tarnawski and Jones, 1998). The down-regulation of EGF-R with ageing by carbohydrate- and fat-rich diets suggests little EGF to perform its protective and healing function. In contrast, the up-regulating role of a protein-rich diet on EGF-R may have increased EGF concentration to stimulate ulcer healing in the present study. In support of this assertion, Reis (2003) suggested that clinicians should adjust the distribution of calories in patients with peptic ulcers.

In conclusion, gastro-aggressive factors increase while gastro-protective factors decrease with age. Furthermore, carbohydrate and fat-rich diets favour the gastro-aggressive factors during ageing. The protein-rich diet facilitates the healing of acetic acid-induced gastric ulcers through enhanced gastric SOD, mucin and EGF-R expression in the ulcerated stomach. Also, protein-rich diets provide improved gastroprotective activity through weakening oxidative and pepsin concentrations and thus may possess anti-inflammatory and anti-secretory properties.

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