Toxicology Reports 7 (2020) 1272–1281

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Age-related changes in haematological parameters and biochemical markers of healing in the stomach of rats with acetic acid induced injury

Ayodeji F. Ajayi [a,b,*], Babafemi S. Olaleye [a]

[a] Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

ARTICLE INFO

Keywords:
Age
Haematology
Neutrophil-lymphocyte ratio
Lipid peroxidation
Re-epithelialisation
Angiogenesis

ABSTRACT

This study examined the changes in haematological and biochemical variables in response to gastric mucosa injury in male Wistar rats divided into four groups according to their ages (3, 6, 12, and 18 months). 0.2 ml of acetic acid was injected intraluminal into the stomach glandular portion of each rat for 45 seconds under anaesthesia. Collection of blood and stomach samples occurred on days 3, 7, 14 and 21 post-induction of gastric ulcer. The results obtained from this study showed 100 % area of gastric mucosa healed in 3-month old rats, 91.72 %, 68.52 % and 62.81 % area of mucosa treated in 6, 12 and 18-month old rats respectively on day 21 post-induction of gastric ulcer. Increased circulation of blood cells in younger rats occurred, neutrophil-lymphocyte ratio (NLR) was decreased in younger rats (3 and 6 months) significantly ($p < 0.05$) when compared to older rats (12 and 18 months). Lipid peroxidation and glutathione (GSH) levels were elevated in older rats (12 and 18 months) significantly ($p < 0.05$) when compared to younger rats (3 and 6 months). In comparison, superoxide dismutase (SOD) and catalase levels were decreased in older rats (12 and 18 months) significantly ($p < 0.05$) when compared to younger rats (3 and 6 months). Histological evaluation showed evidence of early healing with re-epithelialisation and angiogenesis in younger rats, but older rats showed delayed healing. The study showed that the slower rate of healing of gastric ulcer with advancing age in rats might be due to reducing circulating blood cells and anti-inflammatory activities during healing via a lipid peroxidation-dependent mechanism.

1. Introduction

Ageing is a complex process involving a continuous decline of physiological cohesion, as a result of gradual build-up of molecular damage developing into cellular impairment, followed by system and organ damage, which increases the susceptibility to death [1]. The complexity of ageing is highlighted by several hallmarks such as altered intercellular communication, cellular senescence, deregulated nutrient sensing, epigenetic alterations, genomic instability, proteostasis depletion, mitochondria disorder, stem cell exhaustion and telomere attrition [1]. The genetic mechanism regulating the process of ageing is crucial in the quest to counter age-related diseases, particularly cardiovascular diseases, neurodegenerative diseases and cancer [2].

The reactive oxygen species (ROS) are generated throughout the lifespan as a result of the overproduction of free radicals by ageing mitochondria and reduced antioxidant defences [3,4]. Literature has it that ROS, such as superoxide anions, hydrogen peroxide and hydroxyl radicals are involved in the pathogenesis of several human diseases. The diseases include neurodegenerative disorders, viral infections, inflammation, autoimmune pathologies, as well as gastrointestinal pathologies such as gastrointestinal inflammation and gastric ulcer [5,6].

A peptic ulcer is an interruption in the lining of the gastric or duodenal mucosa, which extends to the *muscularis mucosae* and exposes the submucosa [7]. The incidence of many gastrointestinal dysfunctions, including gastric and duodenal ulcers, increases with age [8,9]. These dysfunctions may be due to alteration of the defence and repair

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; ANOVA, Analysis of variance; AST, Aspartate aminotransferase; DTNB, 5, 5' –Dithiobis-2-nitrobenzoic acid; EGF, Epithelial growth factor; GSH, Glutathione; HB, Haemoglobin; KIM-1, Kidney injury molecule-1; MDA, Malondialdehyde; MDA-TBA, Malondialdehyde-thiobarbituric acid; NLR, Neutrophil-lymphocyte ratio; NSAID, Nonsteroidal anti-inflammatory drugs; PDEGF, Platelet-derived endothelial growth factor; PLT, Platelets; RBC, Red blood cell; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TFF, 3 Trefoil factor 3; VEGF, vascular endothelial growth factor; WBC, White blood cell.

* Corresponding author at: Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
E-mail address: aajayi22@lautech.edu.ng (A.F. Ajayi).

https://doi.org/10.1016/j.toxrep.2020.09.007
Received 5 September 2019; Received in revised form 31 August 2020; Accepted 3 September 2020
Available online 19 September 2020

processes, for example, anyone above 60 years of age has a 3-fold increased possibility of developing gastrointestinal complications following the introduction of nonsteroidal anti-inflammatory drugs (NSAIDs) compared to younger individual [10]. Commonly used drugs by the elderly affect the integrity of the gastrointestinal tract. These drugs include; tranquilisers, psychotropic drugs, diuretics, laxatives, antibiotics, and glucocorticoids [10]. Antiulcerogenic drugs, for example, proton pump inhibitors (lansoprazole and omeprazole) tend to cause acute nephritis [11] and hepatitis [12,13], which shows a relationship between gastric ulcer, age, reactive oxygen species and anti-ulcerogenic drugs.

Alanine aminotransferase and aspartate aminotransferase (ALT and AST) and alkaline phosphatase (ALP) are relevant biomarkers of liver functions [14,15]. Increase in the activities of these enzymes in the plasma or serum of rats and mice by acute emotional stress has been suggested to result from damage to various tissues, including gastric tissues [16]. The decrease in ALP activity, in particular, seems to be a general property of all chemicals which are known to provoke severe ulcer, and this decrease is more relevant to peptic ulcer healing than pathogenesis [17].

White blood cells (WBC) are found in various parts of the body, including the blood, lymphoid organs (including lymph nodes, the spleen, Peyer's patches, adenoids and tonsils, and mucosal-associated lymphoid tissue) and other organs of the body [18,19]. Alteration of blood leukocytes, specifically neutrophils, has commonly been attributed to inflammation [20]. Neutrophil to lymphocyte count ratio (NLR) has been proposed as independent predictors of various clinical problems whose pathogenesis involve inflammation [21–23]. Gastric ulcer healing is a genetically programmed repair process. The process includes inflammation, cell proliferation, re-epithelialisation, and formation of granulation tissue, angiogenesis, and scar formation [24]. Therefore markers of inflammation such as NLR and leucocyte counts are important in assessing the gastric ulcer healing process.

Previous studies have shown that natural repair processes of the gastric mucosa are more likely to be completed in relatively shorter time in younger rats when compared with older rats [25]. Therefore this study investigates the age-related changes of some haematological and biochemical variables in the response of the rat gastric mucosa to acetic acid ulceration in an attempt to elucidate further the mechanism by which age alters gastric ulcer healing.

2. Materials and methods

2.1. Experimental animals

The animals used in this experiment were eighty male Wistar rats (ages 3, 6, 12, and 18 months). The rat's age in human years, as reported by previous researchers [26,27] was used in the selection of the groups for this experiment. Three months represent the puberty (12–16 years), six months stand for the period of social maturity (16–20 years), twelve months describe the young adult (25–35 years) and eighteen months are the older adults (40–50 years). Animals were bred (especially to monitor their age) with the parent stock obtained from the Central Animal House, Department of Physiology, University of Ibadan, and Oyo-state, Nigeria. The rats were kept in wire meshed cages and fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) having access to water *ad libitum*. The rats were after that separated into four groups according to their ages each group containing 20 rats. Animals received humane care, and all procedures in this experiment are in accordance to the guiding principles for research on animals as advocated by the Pronouncement of Helsinki and the principles guiding the use and care of animals [28]. The protocol; of the study was approved by the Department of Planning, Research and Statistics Division of the Ministry of Health Oyo-State, Nigeria (Approval Number: AD 13/479/452).

2.2. Acetic acid induced-ulceration

Gastric ulcers were induced using acetic acid following the method described by Ajayi et al. [29] with a little modification. There was no food in the animal's cages 24 h before the induction procedure, and then the stomach of each rat was exposed under anaesthesia [a mixture of xylazine (0.0005 ml/g b.w) and ketamine (0.0015 ml/g b.w)] by performing laparotomy through a midline epigastric incision. The epithelial wall of the stomach clamped with a pair of eye forceps rings, and 0.2 ml of acetic acid (40 % v/v distilled water) injected into the intra-luminal glandular portion of the stomach and withdrawn after 45 s. The stomach was bathed with normal saline to prevent tissue adherence, the abdomen sutured back, and rats were allowed to recover. All the animals received the same feed throughout the experiment, and samples were collected on days 3,7,14 and 21 post inductions. n = 5 and n represent the number of rats used for a particular test (Fig. 1).

2.3. Ulcer area estimation

We determined Gastric Ulcer areas in mm^2 on days 3, 7, 14, and 21 post-induction of ulcer. Five rats were taken from each group on each day and sacrificed by cervical dislocation, and stomach was removed, then opened along the greater curvature, rinsed with normal saline and pinned on a corkboard. Measurement of the macroscopic ulcer area was with the aid of a 2X magnification hand lens. Then the ulcerated area was taken and calculated employing the collection of guiding principles of Drug administration of The Ministry of Health (MOH) Beijing, 1993 as reported by Salami et al. [30] with the formula below:

$$S = \pi\ (d1/2) \times (d2/2),$$

S represents the ulcerated area (mm^2), d1 represents the longest longitudinal diameter of the measured ulcer, and d2 stands for the longest transverse diameter of the measured ulcer.

The percentage of the area of ulcer healed was calculated as described by Adeniyi et al. [31]:

$$\text{Percentage area healed on day7} = \frac{Area\ of\ an\ ulcer\ on\ day3 - the\ area\ of\ an\ ulcer\ on\ day\ 7}{Area\ of\ an\ ulcer\ on\ day\ 3} \times 100$$

$$\text{Percentage area healed on day 14} = \frac{Area\ of\ an\ ulcer\ on\ day3 - the\ area\ of\ an\ ulcer\ on\ day\ 14}{Area\ of\ an\ ulcer\ on\ day\ 3} \times 100$$

$$\text{Percentage area healed on day 21} = \frac{\textit{Area of an ulcer on day}3 - \textit{the area of an ulcer on day } 21}{\textit{Area of an ulcer on day } 3} \times 100$$

2.4. Haematological estimation

Haemoglobin (HB) content, red blood cell count (RBC) white blood cell count (WBC), platelet (PLT) count, and WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophil) were analysed according to the standard techniques described by Baker et al. [32] and Cheesbrough [33]. The neutrophil count was divided by the lymphocyte count to get the Neutrophil-lymphocyte ratio.

2.5. Serum biochemical parameters

Blood samples of each animal collected by cardiac puncture were allowed to clot for 45 min. at room temperature. Serum separated by centrifugation at 4000 rpm for 5 min and analysed for biochemical parameters. The parameters include Serum AST and ALT by the colourimetric method [34]. ALP activity enzymes were determined using Randox Test kits (Randox Laboratories Ltd, Crumlin England, UK) according to the previously described method [35]. Total protein was established by the method described by Brai et al. [36], creatinine was determined using the method described by Barakat, and Mahmoud [37] and urea was estimated using a standard method [38].

2.6. Determination of lipid peroxidation status

Assessment of lipid peroxidation in stomach tissue homogenate was carried out following the procedure described by Varshney, and Kale [39]. This was based upon the reaction of malondialdehyde (MDA) generated as a result of lipid peroxidation with thiobarbituric acid (TBA) producing a pink coloured MDA-TBA adduct that absorbs strongly at 532 nm. MDA was calculated for each sample, as described in a previous report by Ajayi et al. [40].

2.7. Determination of superoxide dismutase

A method initially described by Misra and Fridovich [41] as reported by Magwere et al. [42] was employed. The homogenate was supplemented with 2.5 ml of carbonate buffer, followed by equilibration room temperature; 0.3 ml of 0.3 nM epinephrine solution was then added to the reference and test solution, followed by mixing and reading of absorbance at 420 nm.

2.8. Assay of catalase activity

Catalase activity in the gastric tissue was determined according to the method followed by Öztürk and Demir [43] as reported by Arun et al. [44].

2.9. Assay of total tissue sulfhydryl (thiol) group (reduced glutathione level)

The determination of total tissue sulfhydryl (thiol) group was carried out according to the method reported by Gietler et al. [45]. Gastric tissue was scrapped and homogenised in ice-cold phosphate buffer (pH = 8.0) medium. The tissue homogenate centrifuged at 3,000 rpm for 10 min., and the supernatant collected for the experiment. The tissue supernatant then reacted with 10 mM DTNB (5, 5' –Dithiobis-2-nitrobenzoic acid) of pH 7.0. The resulting suspension was mixed thoroughly and kept at room temperature for 20 min.. The absorbance measured at 412 nm in an ultraviolet-visible spectrophotometer and 2 mM of reduced GSH used as standard.

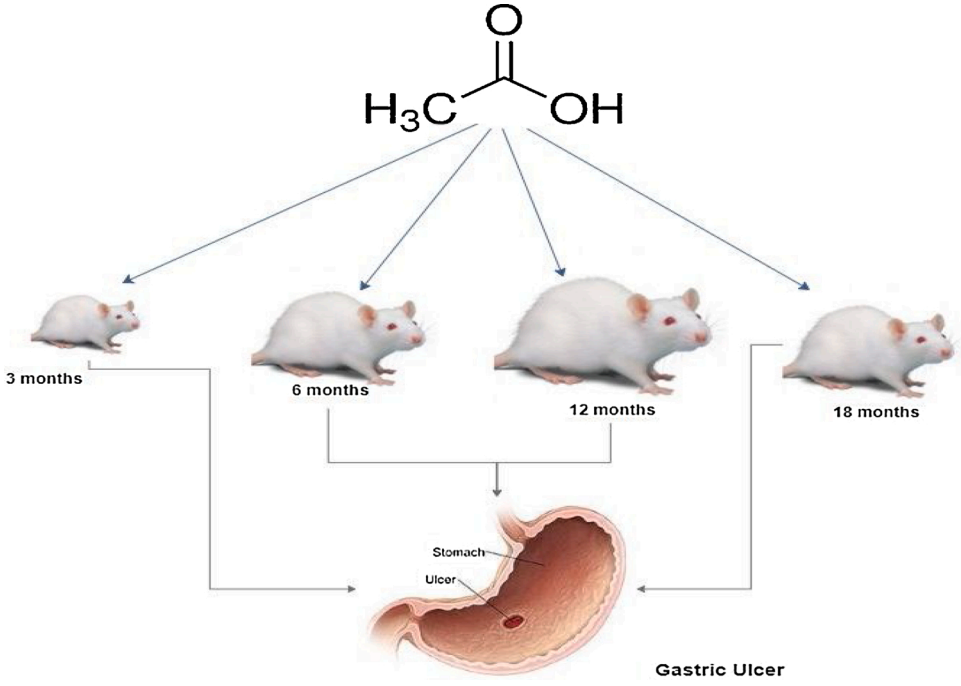

Fig. 1. Schematic diagram of age-related gastric ulcer induction.

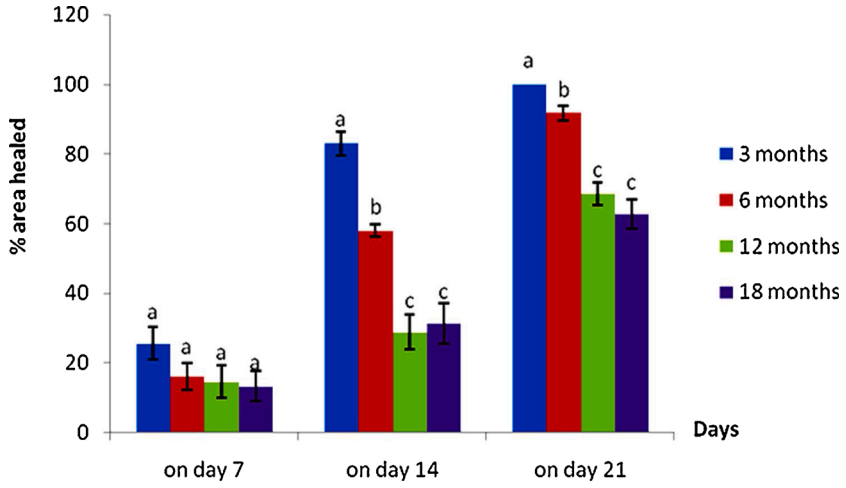

Fig. 2. Age-related changes in the percentage area of ulcer healed on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$.

2.10. Histological processing and examination

The histological study carried out, as described by Akintola [46]. Small sections of the stomach were taken and placed in 10 % formalin for histological analysis. The fixed part cut into five μm sections, after that stained with hematoxylin and eosin. The stained sections assessed for any inflammatory/other pathologic changes, including infiltration of cells, necrosis or damage to the nucleus or tissue structures.

2.11. Statistical analysis

All values presented as Mean ± SEM (standard error of the mean), n = 5, and n stand for the number of rats made use of for a particular study. The statistical significance of the differences among the groups was carried out employing the one-way analysis of variance (ANOVA). Whereas the value of $P < 0.05$ was considered significant.

3. Results

3.1. Effect of age on ulcer area

Fig. 2 showed that the area of gastric mucosa healed in 3-months old rats was significantly greater than that obtained for the other age groups on days 7 and 14. On day 21, the rats aged three months had gastric mucosa which completely healed (100 %), while 6, 12 and 18-month old rats had 91.72 %, 68.52 % and 62.81 % area of mucosa recovered respectively.

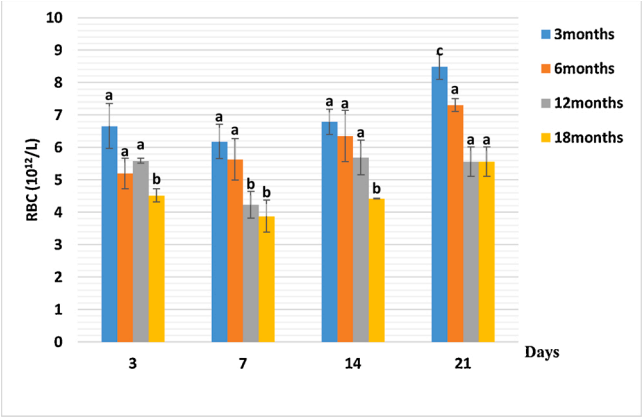

Fig. 3. Age-related changes in the Red Blood Cells concentration of rats on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$.

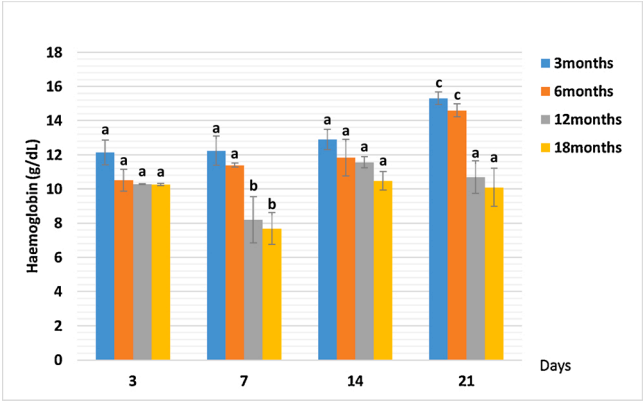

Fig. 4. Age-related changes in the Haemoglobin concentration of rats on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$g/dL = grams per deciliter.

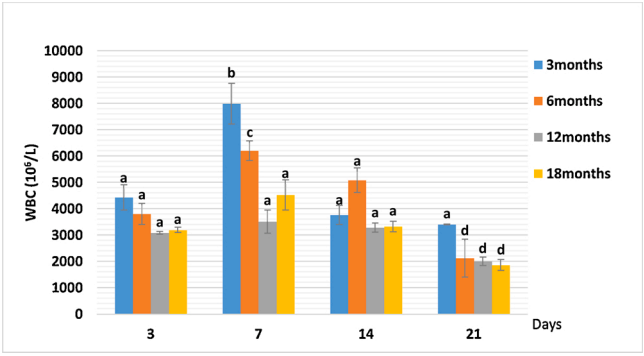

Fig. 5. Age-related changes in White Blood Cells concentration of rats on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$.

3.2. Effect of age on haematological parameters

RBC and PLT counts of younger (3 and 6-month old) rats were

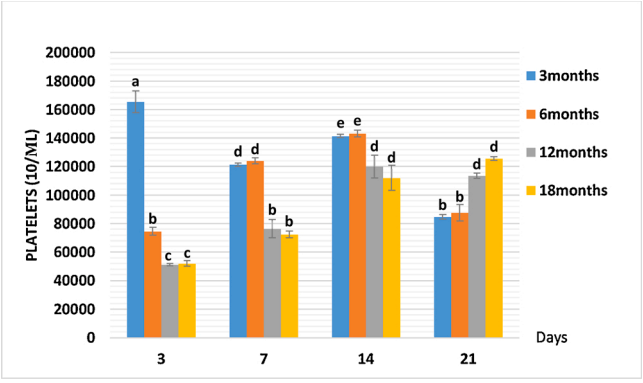

Fig. 6. Age-related changes in the concentration of Platelets on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$.

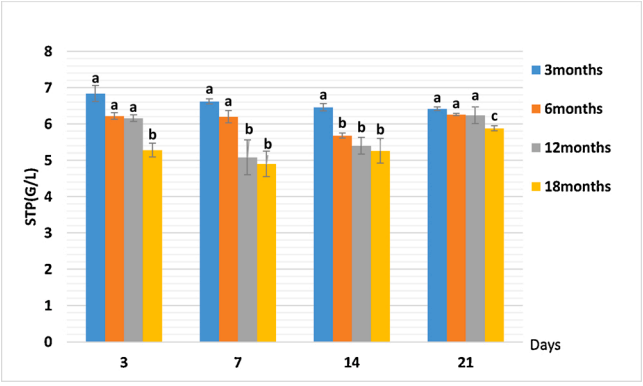

Fig. 7. Age-related changes in Serum Total Protein concentration of rats on days 7, 14, and 21 post-induction of ulcer.
Bars with different alphabets are statistically different at $p < 0.05$.

significantly ($p < 0.05$) higher than that of the older (12 and 18-month old) rats during the healing of the gastric ulcer

(Fig. 3 and Fig. 6). HB contents of younger (3 and 6 months) rats were significantly ($p < 0.05$) higher than that of the older (12 and 18-month old) rats on days 7 and 21 after ulcer induction (Fig. 4). WBC) count decreased significantly ($p < 0.05$) with advancing age of the rats during acetic acid-induced ulcer healing as shown by Fig. 5. NLR reduced with increasing age after induction of ulcer with acetic acid on day 3, and as healing progresses, NLR declined in younger rats (three and six months) when compared to older ones (12 and 18 months) on days 7, 14 and 21 as shown by Table 3.

3.3. Serum Biochemical parameters after induction of gastric ulcer

The total serum protein significantly ($p < 0.05$) decreased in older rats when compared to the younger ones after the induction of ulcer with acetic acid, as shown by Fig. 7.

Activities AST and ALT increased significantly ($p < 0.05$) in older rats compared with younger ones during the healing period. ALP activities decreased significantly ($p < 0.05$) in younger rats for healing to take place and particularly in the 3-month old rats as shown by Table 1.

Serum urea level after induction of gastric ulcer with acetic acid increased with advancing age; this difference was only significant ($p < 0.05$) on day 21. While Serum creatinine level after induction of gastric ulcer with acetic acid significantly ($p < 0.05$) increased with advancing age throughout the healing period as shown by Table 2.

Table 1
Age-related changes in liver enzymes concentration following induction of gastric ulcer with acetic acid.

	Groups	Day 3	Day 7	Day 14	Day 21
AST (IU/L)	**3 months**	37.80 ± 0.49^{a}	39.00 ± 0.63^{b}	37.00 ± 0.63^{a}	36.80 ± 0.49^{a}
	6 months	41.80 ± 0.20^{b}	39.80 ± 0.49^{b}	39.80 ± 0.73^{b}	37.40 ± 1.47^{a}
	12 months	45.20 ± 0.49^{c}	45.20 ± 0.49^{c}	40.20 ± 0.49^{b}	42.00 ± 1.23^{b}
	18 months	44.40 ± 0.75^{c}	44.60 ± 1.40^{c}	40.00 ± 0.63^{b}	40.20 ± 1.02^{b}
ALT (IU/L)	**3 months**	28.20 ± 0.49^{a}	27.00 ± 0.63^{a}	27.80 ± 0.49^{a}	25.20 ± 0.49^{c}
	6 months	31.40 ± 0.25^{b}	29.60 ± 0.98^{a}	28.40 ± 0.98^{a}	27.60 ± 0.98^{a}
	12 months	33.00 ± 0.63^{b}	32.40 ± 0.24^{b}	28.80 ± 0.73^{a}	27.80 ± 0.73^{a}
	18 months	32.80 ± 0.49^{b}	31.60 ± 0.75^{b}	28.60 ± 0.87^{a}	27.80 ± 0.37^{a}
ALP (IU/L)	**3 months**	122.40 ± 0.75^{a}	97.60 ± 1.47^{c}	94.00 ± 0.63^{c}	78.40 ± 1.47^{d}
	6 months	128.40 ± 0.40^{b}	123.20 ± 1.72^{a}	121.20 ± 0.74^{a}	97.00 ± 3.67^{c}
	12 months	123.20 ± 3.60^{a}	112.40 ± 2.69^{a}	116.80 ± 0.49^{a}	109.20 ± 1.96^{a}
	18 months	128.80 ± 0.49^{b}	115.20 ± 4.02^{a}	119.80 ± 1.56^{a}	108.20 ± 2.58^{a}

Figures with different superscripts are significantly different at $P<0.05$.
Legend: Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); Alkaline Phosphatase (ALP).

Table 2
Age-related changes in Creatinine and Urea concentration in serum of rats following induction of gastric ulcer with acetic acid.

	Groups	Day 3	Day 7	Day 14	Day 21
Creatinine (mg/dL)	**3 months**	0.44 ± 0.02^{a}	0.40 ± 0.06^{a}	0.42 ± 0.05^{a}	0.40 ± 0.06^{a}
	6 months	0.68 ± 0.07^{b}	0.76 ± 0.10^{b}	0.56 ± 0.02^{b}	0.62 ± 0.05^{b}
	12 months	0.70 ± 0.63^{b}	0.80 ± 0.03^{b}	0.58 ± 0.04^{b}	0.80 ± 0.06^{b}
	18 months	0.72 ± 0.09^{b}	0.80 ± 0.06^{b}	0.62 ± 0.07^{b}	0.76 ± 0.03^{b}
Urea (mg/dL)	**3 months**	14.60 ± 0.25^{a}	15.00 ± 0.63^{a}	14.20 ± 0.58^{a}	13.00 ± 0.13^{b}
	6 months	15.40 ± 0.25^{a}	15.80 ± 0.49^{a}	15.20 ± 0.58^{a}	14.80 ± 0.20^{a}
	12 months	15.80 ± 0.49^{a}	16.00 ± 0.32^{a}	16.20 ± 0.74^{a}	16.00 ± 0.63^{a}
	18 months	16.00 ± 0.32^{a}	16.20 ± 0.37^{a}	16.80 ± 0.58^{a}	16.40 ± 0.25^{a}

Figures with different superscripts are significantly different at $P<0.05$.

Table 3
Age-related changes in Neutrophil/lymphocyte values of rats following acetic acid-induced gastric ulcer healing.

Groups	Day 3	Day 7	Day 14	Day 21
3 months	0.50 ± 0.02^{a}	0.38 ± 0.03^{c}	0.34 ± 0.02^{c}	0.29 ± 0.03^{d}
6 months	0.46 ± 0.01^{b}	0.38 ± 0.03^{c}	0.38 ± 0.03^{c}	0.33 ± 0.02^{c}
12 months	0.45 ± 0.01^{b}	0.49 ± 0.02^{a}	0.43 ± 0.03^{b}	0.35 ± 0.01^{c}
18 months	0.44 ± 0.00^{b}	0.49 ± 0.02^{a}	0.45 ± 0.02^{b}	0.40 ± 0.03^{c}

Superscripts $^{a, b, c, d}$ = different letters are showing that figures are statistically different among groups at $p < 0.05$).

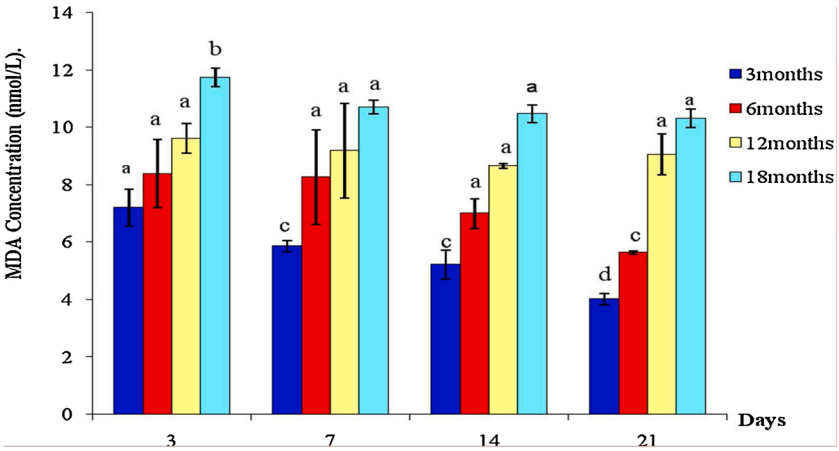

Fig. 8. Malondialdehyde (MDA) concentration (nmol/L) of the rat's stomach during acetic acid-induced gastric ulcer healing.
Bars with different alphabets are statistically different at $p < 0.05$.

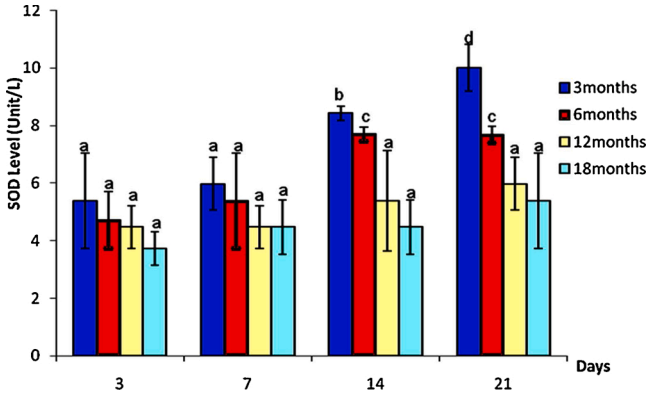

Fig. 9. Superoxide Dismutase (SOD) concentration in rat's stomach during acetic acid-induced gastric ulcer healing (Unit/L).
Bar with different alphabets ars statistically different at $p < 0.05$.

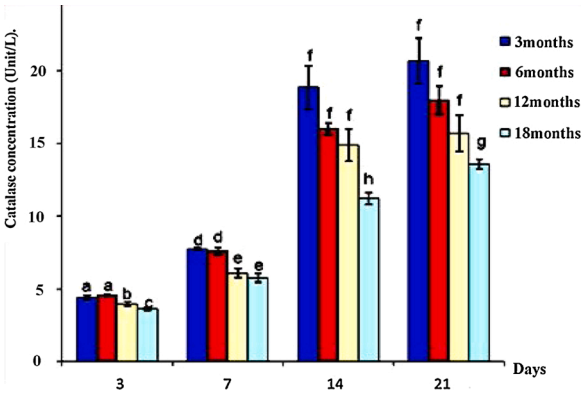

Fig. 11. Catalase concentration (Unit/L) in rat's stomach during acetic acid-induced gastric ulcer healing.
Bars with different alphabets are statistically different at $p < 0.05$.

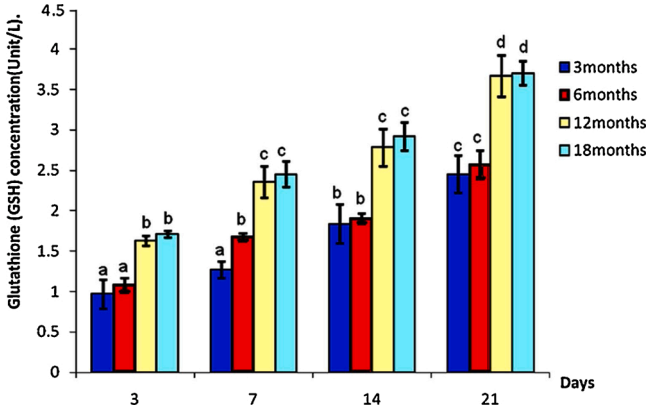

Fig. 10. Glutathione (GSH) concentration of rat's stomach during acetic acid-induced gastric ulcer healing (Unit/L).
Bars with different alphabets are statistically different at $p < 0.05$.

3.4. Effect of age on lipid peroxidation status of stomach tissue during healing of the acetic acid-induced gastric ulcer

Lipid peroxidation decreased in younger rats stomach (3 and 6-month old) by obtaining significantly ($p < 0.05$) lower MDA levels compared with the older rats (12 and 18 months old) concentrations during the healing period (Fig. 8). SOD concentration in rat's stomach decreased significantly ($p < 0.05$) with age on days 14 and 21 post-induction of ulcer (Fig. 9). GSH concentration in rat's stomach increased significantly ($p < 0.05$) with advancing age following the introduction of gastric ulcer (Fig. 10). Catalase concentration in rat's stomach decreased significantly ($p < 0.05$) with advancing age following experimental gastric ulcer with acetic acid (Fig. 11).

3.5. Effect of age on histological examination of the stomach tissues

Histological studies revealed the faster rate of healing in younger rats, showing evidence of healing as early as day 7 and was completed by day 14, signifying early re-epithelialisation and angiogenesis, unlike the delayed ulcer healing observed in older rats.

Histological changes on day 3. The 3-month old rat showed complete ulceration of the mucosal with a bridge of lamina propria, showing necrotic debris and inflammatory cells within the wall. In contrast, the 6-month old rats showed an edge of ulcer with inflammatory cells in the muscular coat and serosa. 12-month old rats showed the side of ulcer with an extensive area of ulceration of the mucosal extending into the muscularis propria. But, the 18-month old rats showed extensive ulceration extending into the muscularis propria (deeper covering more space) defects filled with necrotic debris and inflammatory cells (Fig. 12).

Histological changes on day 7, the 3-month old rat slide showed evidence of regeneration of mucosal. Still, glands are disorderly arranged and residual inflammatory cells seen, the 6-month old rat slide

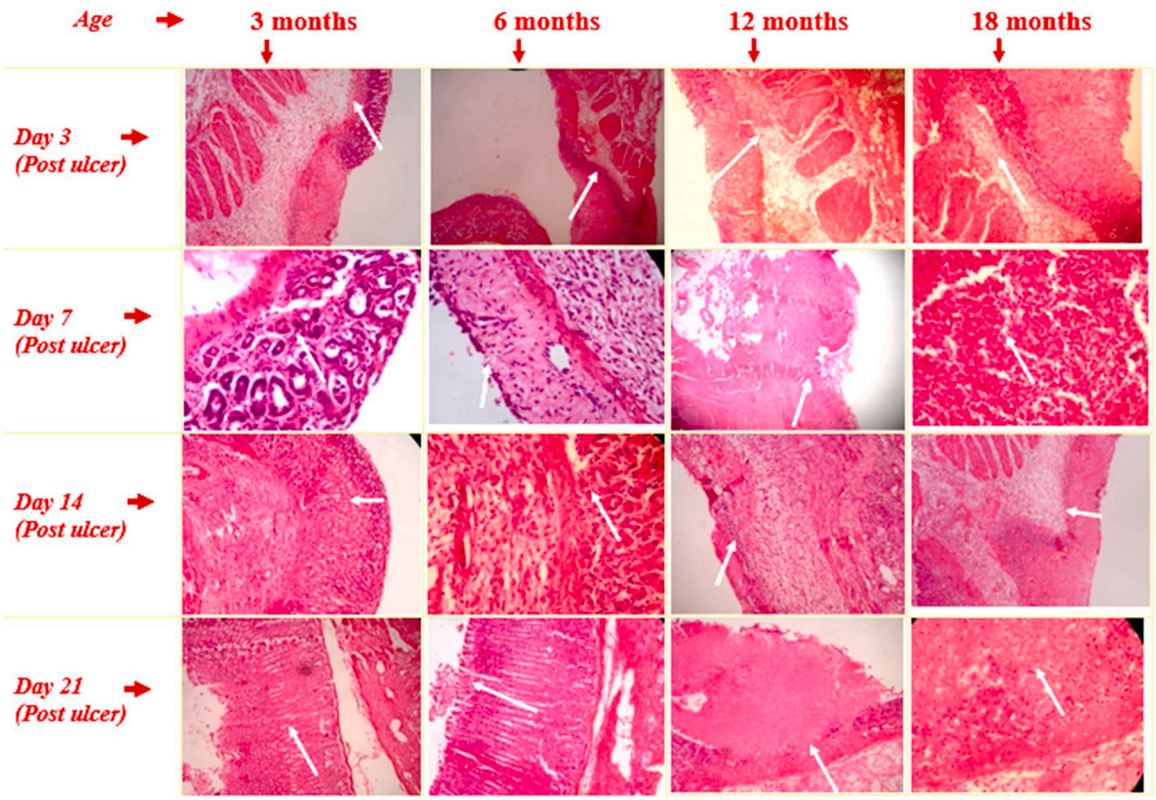

Fig. 12. Photomicrograph showing changes in the histology of the gastric mucosa post gastric ulcer induction (H&E STAIN, MAG. × 100). White arrow = Points to the changes described for each slide.

showed a single layer of regenerating epithelial cells, showing evidence of re-epithelialisation. The 12-month old rat is showing the ulcer crata filled with inflammatory cells and proliferating capillaries with no evidence of re-epithelisation, and the 18-month old rats showed the edge of ulcer, showing numerous polymorphs and proliferating capillaries (Fig. 12).

Histological changes on day 14, the 3-month old rat slide showed the full thickness of normal mucosa and submucosa. The 6-month old rat slide is showing residual inflammatory (polymorphs) cells in the mucosa and submucosa. The stomach of 12-month old rat showed ulceration of the mucosal, bridge of lamina propria, necrotic debris and few granulation tissues at the surface. The 18-month old rat showed ulceration of the mucosa coat and serosa, with defects filled with inflammatory cells (Fig. 12).

Histological changes on day 21, The 3 and 6-month old rat slides showing the full thickness of normal mucosa and submucosa. The 12-month old rat slide was showing little evidence of healing but a significant reduction in the number of inflammatory cells. The 18-month old rat slide was showing ulcer crata filled with necrotic debris and few inflammatory cells (Fig. 12).

4. Discussion

Applications of acetic acid into the intra-luminal glandular portion of the stomach of rat resulted in ulcers of consistent size and severity at an incidence of 100 %, and highly resemble human gastric ulcer in terms of both pathological features and healing mechanisms. Reduced rate of healing with increasing age was observed in this study as reported earlier by Ajayi and Olaleye [25].

The decision on specific treatment for gastric and duodenal ulcers by the physician depends on certain factors such as patient's age, overall health status, medical history, the intensity of pathogenesis and tolerance for medications [47]. Physiological investigations have shown that stress from any source can affect the endocrine, hemopoietic and immune systems [48,49]. Various studies have shown that erythrocytes, neutrophils, and platelets count increased, whereas lymphocytes, eosinophils, and monocytes decrease in number as a result of stress [50, 51]. Considering the relationship between oxidative stress and age, it, therefore, become pertinent to investigate the changes in some haematological and biochemical variable during the healing of gastric ulcer in rats of different ages.

Red blood cell and haemoglobin are essential in the process of healing of the ulcer; haemoglobin resides in the red blood cell. It is responsible for the amount of oxygen carried by the blood which in turn gives the amount that will be delivered to the tissue, oxygen availability affects all phases of healing and is therefore vital for healing to take place [52]. The reduction in Red blood cell count and haemoglobin concentration from the administration of aspirin have been observed previously in gastric tissue damage [52]. In this study, Red blood cell count and haemoglobin concentration increased in younger rats, making more oxygen available for the formation of granulation tissue resulting in faster healing.

The body's cellular defence mechanisms recruited by a biological response to the damaged area, accompanied by vascular and neural responses are essential in tissue repair [53]. Previous studies showed that the natural healing process in the rats would require an immune response booster through increased white blood cell production [54]. This study observed that the White blood cell counts increased in younger rats, unlike the older ones after ulcer induction with acetic acid, probably suggesting a higher immune response in younger animals.

Platelets are vital for wound healing by releasing some growth factors that can promote angiogenesis through the release of pro- and anti-angiogenic elements [55,56]. Formidable angiogenic stimulators such as; vascular endothelial growth factor (VEGF), Platelet-derived endothelial growth factor (PDEGF), Epithelial growth factor (EGF) and PDEGF stored in platelets, account for the ability of platelets to stimulate

endothelial cell proliferation and capillary-like formation [57]. The increase in platelets count in younger rats after the induction of ulcer might be a signal of enhanced angiogenesis process, which in turn accelerates ulcer healing.

Alkaline phosphatase enzyme plays an essential role in tissue necrosis associated with various gastrointestinal ulceration models such as; absolute alcohol and aspirin-induced ulcer while increases in its (ALP) activities may occur in damaged tissues [58]. The decreased ALP activity is one of the first lines of defence by which the gastroduodenal mucosa and the mucous bicarbonate barrier overlying the gastric epithelium protect itself [59]. During healing in this study, the activities of these enzymes reduced in younger rats with that of the older rats became elevated. This observed reduction in the more immature rats might be another mechanism by which there was prompt or enhanced ulcer healing reported in earlier studies [25]. Increased level of serum urea and serum creatinine also reflects cellular damage [60]. While, the concentration of new urinary biomarkers such as kidney injury molecule-1 (KIM-1), calbindin, and trefoil factor 3 (TFF3) have been used to monitor the progression of kidney damage induced by cisplatin treatment in cancer patients [61]. The results of this investigation suggest that reductions in urea and creatinine levels seen in younger rats are indications of reduced cellular damage. Also could lead to enhance gastric ulcer healing, which may also be contributing to the faster healing rate seen in 3 and 6- month old rats.

Neutrophil to lymphocyte ratio (NLR) is an indicator of inflammatory status [62,63], during disease conditions. Recently, the NLR has been documented to be increased in inflammatory disorders like pancreatitis and ulcerative colitis [64]. Increases in NLR indicate it more inflammation occurring, while a decrease suggests reduced inflammation, which gives rise to improved healing. In this study, as healing progressed, younger rats maintained a lower NLR, unlike older rat, which suggests a faster cure. The increased inflammation with age may be due to the biological processes put in place to remove accumulated damaged proteins due to increased oxidative stress in old age; this stimulates inflammatory responses leading to chronic inflammatory state [65,66].

Lipid peroxidation is an activity through which oxidants such as free radicals attack (membrane) lipids containing carbon-carbon double bond(s) such as polyunsaturated fatty acids [67]. Increased lipid peroxidation and breakage of fats result in the formation of reactive compounds leading to alterations in the membrane lipid bilayer permeability and fluidity, which affect cell integrity as a result of oxidative stress [68]. Thus during gastritis and gastric ulcers, stress is inevitable [69], possibly as a result of increased lipid peroxidation. This study reveals that gastric tissue of younger rats prevents lipid peroxidation process, resulting in higher enzymatic antioxidant [superoxide dismutase and Catalase] levels. These results further shed light on the faster rate of healing observed in younger rats in earlier studies by Ajayi and Olaleye [25]. It also confirms the role played by increased oxidative stress in the healing rate of gastric ulcer of older rats by earlier researchers [65]. Oxidative stress has been documented to be raised in elderly subjects, possibly as a result of uncontrolled free radicals production by the ageing mitochondria as well as decreased antioxidant defences [70].

Histological observations revealed early re-epithelialisation of gastric mucosa and angiogenesis in younger rats. According to Tarnawski et al. [71], ulcer re-epithelialisation is an essential process for gastrointestinal ulcer healing, which without restoration of a continuous epithelial barrier, the mucosa becomes vulnerable to mechanical or chemical injury and infections thereby preventing ulcer healing. Earlier reports found that reduced neutrophil infiltration into ulcerated gastric tissues has been implicated in the promotion of acetic acid-induced chronic ulcer healing in rats [72]. This study is also in agreement with these earlier findings.

5. Conclusion

The study shows that the slower rate of healing of gastric ulcer with age in rats might be due to the reducing circulating blood cells and anti-inflammatory activities during healing via a lipid peroxidation-dependent mechanism.

6. Recommendations

Synolytic drugs, metformin, rapamycin and spermidine with anti-ageing properties as well as nicotinamide riboside capable of repairing DNA damage, cellular signaling pathways, and eventually improving cell physiology could be explored to enhance gastric mucosa healing in older rats in subsequent studies [73].

Also, non-pharmacological anti-ageing interventions, like lifestyle adjustment, caloric restriction, consumption of fruits and vegetables rich in antioxidants, intermittent fasting, and exercise [72,74,75] can be a consideration for consideration in the quest for the solution to the delayed gastric ulcer healing in older individuals.

Therefore, it is recommended that future studies should be focused on making up for the disparity in the healing rate between the older and younger rats by investigating the potentials of the drugs mentioned above in promoting gastric ulcer healing in older rats.

Funding

None.

CRediT authorship contribution statement

Ayodeji F. Ajayi: Investigation, Writing - original draft. **Babafemi S. Olaleye:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.09.007.

References

[1] C. López-Otín, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging, Cell 153 (6) (2013) 1194–1217, https://doi.org/10.1016/j.cell.2013.05.039.
[2] P.S. Param, A.D. Brittany, D.N. Ravi, B. Anne, The genetic of ageing: a vertebrate perspective, Cell 177 (1) (2019) 200–220, https://doi.org/10.1016/j.cell.2019.02.038.
[3] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature 408 (6809) (2000) 239–247, https://doi.org/10.1038/35041687.
[4] V.B. Junqueira, S.B. Barros, S. Chan, L. Rodrigues, L. Giavarotti, R. Abud, G. Deucher, Ageing and oxidative stress, Mol. Aspects Med. 25 (1-2) (2004) 5–16, https://doi.org/10.1016/j.mam.2004.02.003.
[5] M.G. Repetto, S.F. Llesuy, Antioxidant properties of natural compounds used in popular medicine for gastric ulcers, Braz. J. Med. Biol. Res. 35 (5) (2002) 523–534, https://doi.org/10.1590/s0100-879x2002000500003.
[6] S. Dhanalakshmi, R. Srikumar, S. Manikandan, N.J. Parthasarathy, R.S. Devi, Antioxidant property of Triphala on cold stress-induced oxidative stress in experimental rats, J. Health Sci. 52 (2006) 843–847, https://doi.org/10.1248/jhs.52.843.
[7] C. DeGiacomo, P.L. Bacchini, M. Lombardi, La patologiagastrica, in: G.L. De Angelis (Ed.), L'endoscopiaDigestiva in etàPediatrica e Giovanile, EMSI, Rome, Italy, 2002, pp. 49–68.
[8] K. Thorsen, J.A. Soreida, J.T. Kyaloy, T. Glomsaker, K. Soreide, Epidemiology of perforated peptic ulcer: age- and gender-adjusted analysis of incidence and mortality, World J. Gastroenterol. 19 (3) (2013) 347–354, https://doi.org/10.3748/wjg.v19.i3.347.
[9] S.Y.T. Raymond, C.Y. Justin, Managing peptic ulcer and gastroesophageal reflux disease in elderly Chinese patients – focus on esomeprazole, Clin. Interv. Aging 8 (2013) 1433–1443, https://doi.org/10.2147/CIA.S41350.

[10] M.T.F.D.S. Campos, J.B.R. Monteiro, A.P.R.D.C. Ornelas, Factors that affect the aged people food intake and nutrition, Revista de Nutricao 13 (2000) 157–165.
[11] B.R. Alan, D.S. Michel, K. Narmin, K. Holly, Safety of the long-term use of proton pump inhibitors, World J. Gastroenterol. 16 (19) (2010) 2323–2330, https://doi.org/10.3748/wjg.v16.i19.2323.
[12] D. Alhankawi, S. Sharma, K. Sun, N. Theise, J. Park, Proton pump inhibitor-induced liver injury, Am. J. Gastroenterol. 113 (2018) S1349–S1350.
[13] V. Domínguez-Leñero, M. Barrera-Ledesma, M. Romero-Alonso, M.T. Garrido Martínez, Stevens-Johnson syndrome and toxic hepatitis due to esomeprazole, Farm Hosp. 33 (2) (2009) 118–119, https://doi.org/10.1016/s1130-6343(09)71002-5.
[14] G. Kasarala, H.L. Tillmann, Standard liver tests, Clin. Liver Dis. 8 (1) (2016) 13–18, https://doi.org/10.1002/cld.562.
[15] M.R. McGill, The past and present of serum aminotransferases and the future of liver injury biomarkers, EXCLI J. 15 (2016) 817–828, https://doi.org/10.17179/excli2016-800.
[16] O. Sanchez, A. Amau, M. Pareja, E. Poch, I. Ramirez, M. Soley, Acute stress-induced tissue injury in mice; differences between emotional and social stress, Cell Stress Chaperones 7 (1) (2002) 36–46, https://doi.org/10.1379/1466-1268(2002)007<0036:asitii>2.0.co;2.
[17] S.K. Das, C. Roy, The protective role of Aegle marmelos on aspirin-induced gastroduodenal ulceration in an albino rat model: possible involvement of antioxidants, Saudi J. Gastroenterol. 18 (3) (2012) 188–194, https://doi.org/10.4103/1319-3767.96452.
[18] T.D. Randall, D.M. Carragher, J. Rangel-Moreno, Development of secondary lymphoid organs, Annu. Rev. Immunol. 26 (2008) 627–650, https://doi.org/10.1146/annurev.immunol.26.021607.090257.
[19] N.H. Ruddle, E.M. Akirav, Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response, J. Immunol. 183 (4) (2009) 2205–2212, https://doi.org/10.4049/jimmunol.0804324.
[20] R. Nagatomi, The implication of alterations in leukocyte subset counts on immune function, Exerc. Immunol. Rev. 12 (2006) 54–71.
[21] S.M.A. Muhammed, J.A.A. Bahnacy, A.A.A. Ali, A.V. Pathare, S.S. Alkindi, Predictive value of neu¬trophil to lymphocyte ratio in outcomes of patients with the acute coronary syndrome, Arch. Med. Res. 41 (8) (2010) 618–622, https://doi.org/10.1016/j.arcmed.2010.11.006.
[22] G. Ouellet, R. Malhotra, E.L. Penne, L. Usvya, N.W. Levin, P. Kotanko, Neutrophil-lymphocyte ratio as a novel predictor of survival in chronic hemodialysis patients, Clin. Nephrol. 85 (4) (2016) 191–198, https://doi.org/10.5414/CN108745.
[23] H.K. Jae, Y.L. Jun, K.K. Hae, W.L. Jin, G.J. Sung, J. Kyoungwon, E.K. Sung, M. Won, I.P. Moo, J.P. Seun, Prognostic significance of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in patients with stage III and IV colorectal cancer, World J. Gastroenterol. 23 (3) (2017) 505–515, https://doi.org/10.3748/wjg.v23.i3.505, 21.
[24] H.J. You, S.K. Han, Cell therapy for wound healing, J. Korean Med. Sci. 29 (3) (2014) 311–319, https://doi.org/10.3346/jkms.2014.29.3.311.
[25] A.F. Ajayi, S.B. Olaleye, Age-related changes in the response of the rat gastric mucosa to acetic acid- and indomethacin-induced ulceration, Arch. Bas. App. Med. 3 (2015) 79–88.
[26] J.D. Iandoli, A.J.T. Nigro, A. Sementilli, Y. Juliano, N.F. Novo, End-to-end esophagogastric anastomosis comparative study, between a single layer and submucosa-mucosa invagination technics: in rats, Acta Cir. Bras. 15 (1) (2000) 14–22.
[27] P. Sengupta, The laboratory rat: relating its age with human's, Int. J. Prev. Med. 4 (6) (2013) 624–630.
[28] World Medical Association, American Physiological Society (WMA, APS), guiding principles for research involving animals and human beings, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2) (2002) R281–R283, https://doi.org/10.1152/ajpregu.00279.2002.
[29] A.F. Ajayi, A.M. Folawiyo, T.A. Salami, Ulcer healing mechanism of ethanolic extract of Talinum triangulare in male wistar rats, Unravelled Knowledge J. Pharm. Biosci. 4 (2016) 41–50.
[30] A.T. Salami, N.G. Ndukauba, T.O. Iyiola, O.F. Agbola, F.S. Oluwole, S.B. Olaleye, Gastroprotective properties of manganese chloride on acetic acid-induced ulcer in wistar rats afr, J. Biomed. Res. 17 (2) (2014) 109–117.
[31] O.S. Adeniyi, J. Omale, R.I. Egwuje, O.S. Ajayi, Effects of selenium treatment on healing of acetic acid-induced gastric ulcer in albino wistar rats, Am. J. Biomed. Res. 4 (1) (2016) 18–22, https://doi.org/10.12691/ajbr-4-1-4.
[32] F.J. Baker, R.E. Silverton, C.J. Pallister, Baker and Silverton's Introduction to Medical Laboratory Technology, seventh ed., 1998, pp. 356–360.
[33] M. Cheesbrough, District laboratory practices in tropical countries part 2, Low price edition (2000) 267–334.
[34] R. Edris, Analysis of antioxidants and serum biochemical responses in goldfish under nickel exposure by sub-chronic test, J. Appl. Anim. Res. 45 (1) (2017) 320–325, https://doi.org/10.1080/09712119.2016.1190732.
[35] S.Y. Hasan, A. Aftab, M.Z. Ahmed, Isolation of Astragaloside-IV and Cyclocephaloside-1 from Astragalus gummifera and evaluation of Astragaloside-IV on CCl4 induced liver damage in rats, Asian J. Biol. Sci. 8 (2015) 1–15, https://doi.org/10.3923/ajbs.2015.1.15.
[36] B.I.C. Brai, J.A. Folode, R.A. Adisa, A.A. Odetola, Effects of aqueous leaf extract of avocado (Persea americana) on total cholesterol, triacylglycerol, protein and haematological parameters in CCl4-intoxicated rats, Clin. Phytoscience 6 (1) (2020) 4, https://doi.org/10.1186/s40816-020-00159-y.
[37] L.A. Barakat, R.H. Mahmoud, The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemia rats, N. Am. J. Med. Sci. 3 (9) (2011) 411–417, https://doi.org/10.4297/najms.2011.3351.
[38] M.A. Kandeil, K.M. Hassanin, M.M. Arafa, H.A. Abdulgawad, G.M. Safwat, Pomegranate peels ameliorate renal nitric oxide synthase, interleukin-1β and, and Kidney injury molecule-1 in nephrotoxicity induced by acrylamide in rats, Egypt Pharmaceut. J. 18 (4) (2019) 368–376, https://doi.org/10.4103/epj.epj_25_19.
[39] R. Varshney, R.K. Kale, Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes, Int. J. Radiat. Biol. 58 (5) (1990) 733–743, https://doi.org/10.1080/09553009014552121.
[40] A.F. Ajayi, R.E. Akhigbe, L.O. Ajayi, Lipid peroxidation and antioxidant enzymes activities in the kidneys of experimental dysthyroid rabbits: an assessment of renal redox state, Int. J. Infect. Trop. Dis. 5 (2019) 16–23.
[41] H.P. Misra, I. Fridovich, The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 (10) (1972) 3170–3175.
[42] T. Magwere, Y.S. Naik, T.A. Hasler, Effects of chloroquine treatment on antioxidants enzymes in rat's liver and kidney, Free Rad Biol Med. 22 (1-2) (1997) 321–327, https://doi.org/10.1016/s0891-5849(96)00285-7.
[43] L. Öztürk, Y. Demir, In vivo and in vitro protective role of proline, Plant Growth Regul. 38 (3) (2002) 259–264, https://doi.org/10.1023/A:1021579713832.
[44] A. Ray, S.R. Chaudhuri, B. Majumdar, S.K. Bandyopadhyay, Antioxidant activity of ethanol extract of rhizome of Picrorhiza kurroa on indomethacin-induced gastric ulcer during the healing, Indian J. Clin. Biochem. 17 (2) (2002) 44–51, https://doi.org/10.1007/BF02867970.
[45] M. Gietler, M. Nykiel, B.M. Zagdanska, Changes in the reduction state of ascorbate and glutathione, protein oxidation and hydrolysis leading to the development of dehydration intolerance in Triticum aestivum L. Seedlings, Plant Growth Regul. 79 (2016) 287–297, https://doi.org/10.1007/s10725-015-0133-z.
[46] A.O. Akintola, B.D. Kehinde, J.O. Fakunle, A.F. Ajayi, Synergistic and ameliorative effect of honey and coconut water on crude oil induced toxicity in rats, Res. J. Environ. Toxicol. 12 (1) (2018) 24–33, https://doi.org/10.3923/rjet.2018.24.33.
[47] University of Chicago Medical Centre Website, Online Library (UCMC), Digestive Disorder; Stomach and Duodenal Ulcer (Peptic Ulcer), The University of Chicago Medical centre, 2013. www.uchospitals.edu/online-library/content=p00394.
[48] M. Maes, M. Vander Planken, A. Van Gastel, Influence of academic stress on haematological measurements in subjectively healthy volunteers, Psychiatry Res. 80 (1998) 201–212.
[49] D. Benoit, L. Esa, G. Ralph, The driving test as a stress model: effects on blood picture, serum cortisol and the production of interleukins in man, Life Sci. 68 (14) (2001) 1641–1647, https://doi.org/10.1016/s0024-3205(01)00963-8.
[50] F.S. Dhabhar, A.H. Miller, B.S. McEwen, R.L. Spencer, Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms, J. Immunol. 154 (10) (1995) 5511–5527.
[51] R.H. Asmah, G. Yeboah, H. Asare-Anane, S. Antwi-Baffour, T.N. Archampong, C. A. Brown, G. Amegatcher, D.N. Adjei, B. Dzudzor, J. Akpalu, P.F. Ayeh-Kumi, Relationship between oxidative stress and haematological indices in patients with diabetes in the Ghanaian population, Clin. Diabetes Endocrinol. 1 (7) (2015), https://doi.org/10.1186/s40842-015-0008-2.
[52] M.A. Adefisayo, R.O. Akomolafe, S.O. Akinsomisoye, et al., Gastroprotective effect of methanol extract of Vernonia amygdalina (del.) leaf on aspirin-induced gastric ulcer in Wistar rats, Toxicol. Rep. 4 (2017) 625–633, https://doi.org/10.1016/j.toxrep.2017.11.004.
[53] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (6) (2010) 805–820, https://doi.org/10.1016/j.cell.2010.01.022.
[54] V.T. Paul, M. Christopher, E. George, O. Enow, D. Theophile, N. Barthelemy, Healing effect on chronic gastric ulcers and short-term toxicity profile of the leaf methanol extract of Ocimum suave wild (Lamiaceae) in rats, African Journal, Afr. J. Tradit. Complement. Altern. Med. 2 (3) (2005) 312–325.
[55] L. Ma, S.N. Elliott, G. Cirino, A. Buret, L.J. Ignarro, J.L. Wallace, Platelets modulate gastric ulcer healing: role of endostatin and vascular endothelial growth factor release, Proc. Natl. Acad. Sci. U.S.A. 98 (11) (2001) 6470–6475, https://doi.org/10.1073/pnas.111150798.
[56] E.M. Constanza, C.S. Patricio, A.A. Verónica, The influence of platelet-derived products on angiogenesis and tissue repair: a brief update, Front 6 (2015) 290, https://doi.org/10.3389/fphys.2015.00290.
[57] R. Perini, W. McKnight, M. Dicay, A. Klein, M.D. Hollenberg, J.L. Wallace, Roles of platelets and proteinase-activated receptors in gastric ulcer healing, Dig. Dis. Sci. 50 (2005) S12–S15, https://doi.org/10.1007/s10620-005-2801-8.
[58] E. Mornet, E. Stura, A.S. Lia-Baldini, T. Stigbrand, A. Ménez, M.H. Le Du, Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralisation, J. Biol. Chem. 276 (2001) 31171–31178, https://doi.org/10.1074/jbc.M102788200.
[59] G. Flemstrom, E. Kivilaakso, Demonstration of a pH gradient at the luminal surface of rat duodenum in vivo and its dependence on the mucosal alkaline secretion, Gastroenterology 84 (4) (1993) 787–794.
[60] O.A. Sodipo, F.I. Abdulrahman, U.K. Sandabe, Biochemical kidney function with aqueous fruit extract of Solanum macrocarpum (Linn) in albino rats chronically administered Triton-X to induce hyperlipidemia, AJMMS 3 (2) (2012) 93–98.
[61] B. George, X. Wen, N. Mercke, et al., Time-dependent changes in kidney injury biomarkers in patients receiving multiple cycles of cisplatin chemotherapy, Toxicol. Rep. 7 (2020) 571–576, https://doi.org/10.1016/j.toxrep.2020.04.003.
[62] K.J. Halazun, A. Aldoori, H.Z. Malik, A. Al-Mukhtar, K.R. Prasad, G.J. Toogood, J. P.A. Lodge, Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases, Eur. J. Surg. Oncol. 34 (1) (2008) 55–60, https://doi.org/10.1016/j.ejso.2007.02.014.

[63] A. Jafarzadeh, V. Akbarpoor, V. Nabizadeh, M. Nemati, M.T. Rezayati, Total leukocyte counts and neutrophil-lymphocyte count ratios among Helicobacter pylori-infected patients with peptic ulcers: independent of the bacterial status, Southeast Asian J. Trop. Med. Public Health 44 (1) (2013) 82–88.
[64] B.N. Coşkun, M.F. Öksüz, S. Ermurat, A.N. Tufan, N. Oruçoğlu, A. Doğan, E. Dalkılıç, Y. Pehlivan, Neutrophil lymphocyte ratio can be a valuable marker in defining disease activity in patients who have started antitumor necrosis factor (TNF) drugs for ankylosing spondylitis, Eur. J. Rheumatol. 1 (3) (2014) 101–105, https://doi.org/10.5152/eurjrheumatol.2014.034.
[65] E.S. Cannizzo, C.C. Clement, R. Sahu, C. Follo, L. Santambrogio, Oxidative stress, inflammation-ageing and immunosenescence, J. Proteomics 74 (11) (2011) 313–2323, https://doi.org/10.1016/j.jprot.2011.06.005.
[66] M. Celikbilek, S. Dogan, O. Ozbakır, G. Zararsız, H. Kücük, S. Gürsoy, A. Yurci, K. Güven, M. Yücesoy, Neutrophil-lymphocyte ratio as a predictor of disease severity in ulcerative colitis, J. Clin. Lab. Anal. 27 (1) (2013) 72–76, https://doi.org/10.1002/jcla.21564.
[67] A. Antonio, F.M. Mario, A. Sandro, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nominal, Oxid. Med. Cell. Longev. 360438 (2014), https://doi.org/10.1155/2014/360438, 31 pages.
[68] C. Berasain, J. Castillo, M.J. Perugorria, M.U. Latasa, J. Prieto, M.A. Avila, Inflammation and liver cancer: new molecular links, Ann. N. Y. Acad. Sci. 1155 (2009) 206–221, https://doi.org/10.1111/j.1749-6632.2009.03704.x.
[69] V. Kumar, A. Abbas, N. Fausto, Stomach. Pathologic Basis of Diseases, 8th ed., Elsevier Saunder, 2007, pp. 833–890.
[70] R. Anu, K. Amit, S. Vivek, Y. Brijes, T. Ruchi, C. Sandip, D. Kuldeep, Oxidative Stress, Prooxidants, and Antioxidants: The Interplay, Biomed Res Int., 2014, https://doi.org/10.1155/2014/761264.
[71] A. Tarnawski, I.L. Szabo, S.S. Husain, B. Soreghan, Growth factors and signal transduction pathways trigger regeneration of gastric mucosa during ulcer healing, J. Physiol. 95 (1–6) (2001) 337–344, https://doi.org/10.1016/s0928-4257(01)00046-8.
[72] T. Kobayashi, Y. Ohta, J. Yoshino, S. Nakazawa, Teprenone promotes the healing of acetic acid-induced chronic gastric ulcer in rats by inhibiting neutrophils infiltration and lipid peroxidation in ulcerated gastric tissue, Pharmacol. Res. 43 (1) (2001) 23–30, https://doi.org/10.1006/phrs.2000.0748.
[73] M. Gonzalez-Freire, A. Diaz-Ruiz, D. Hauser, J. Martinez-Romero, L. Ferrucci, M. Bernier, R. Carbo, The road ahead for health and lifespan interventions, Ageing Res. Rev. (2020) 59, https://doi.org/10.1016/j.arr.2020.101037.
[74] D.S. Hwangbo, H.Y. Lee, L.S. Abozaid, K.J. Min, Mechanisms of lifespan regulation by calorie restriction and intermittent fasting in model organisms, Nutrients 12 (4) (2020) 1194, https://doi.org/10.3390/nu12041194.
[75] M. Ji, X. Gong, X. Li, C. Wang, M. Li, Advanced research on the antioxidant activity and mechanism of polyphenols from Hippophae species-a review, Molecules 25 (4) (2020) 917, https://doi.org/10.3390/molecules25040917.