ORIGINAL PAPER

Nig. J. Pharm. Res. 2016, 12 (2) pp 189-206
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

Role of Nitric Oxide and Endogenous Antioxidants in Thyroxine Facilitated Healing of Ischemia-Reperfusion Induced Gastric Ulcers

A. T. Salami[B-F], O. A. Odukanmi[B-F], C. O. Olagoke[B-F], T. O. Iyiola[B-F] and *S. B. Olaleye[A-F]

Gastrointestinal Secretions and Inflammatory Research Unit, Department of Physiology, College of Medicine, University of Ibadan, Ibadan.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Studies have revealed the role of thyroxine during healing of gastric ulcers with information lacking on the mechanism involved hence the focus of this study.
Materials and Methods: Adult male Wistar rats (150 – 200g) were randomly divided into 4 groups (n=5 per group): Normal control (NC), Sham ulcerated (SU), Thyroidectomised ulcerated untreated (ThU) and Thyroidectomised ulcerated + Levo-thyroxine (100µg/kg/day) (ThU + T_4). Animals were stabilised for 35 days following thyroidectomy and treated accordingly to experimental groupings. Weekly body weight changes were recorded, gastric ulcer was induced by ischemia-reperfusion and gastric acid secretion evaluated. They were sacrificed 1 hour, 3 and 7 days post ulcer induction, blood samples collected for haematological indices through cardiac puncture and their stomachs prepared for gross and microscopic examinations to assess gastric healing. Gastric tissue protein, malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), and Nitric oxide (NO) were assessed as biomarkers of healing. Data were analysed using one way ANOVA and Student's t test with $p< 0.05$ considered statistically significant.
Results: Thyroxine treated rats showed significant weight loss compared with NC and ThU groups. Percentage healing rate was significantly increased in thyroxine treated group compared with ThU animals by 1 hour (42.45% and -42.81%), days 3 (35.14% and -59.36%), and 7 (64.29% and -115.7%). Hematological indices significantly increased in thyroxine treated group compared with other groups. Thyroxine treatment significantly reduced Neutrophil/Lymphocyte; Platelet/NO as well as lipid peroxidation index in this study. Superoxide dismutase, CAT and NO increased significantly in thyroxine treated rats compared with other groups.
Conclusion: Thyroxine treatment facilitates the healing of ischeamic-reperfused gastric ulcers possibly by increasing NO activity which in turn causes increased vasodilatation and enhanced endogenous antioxidants at the ulcer sites.
Keywords: Nitric Oxide, Antioxidants, Thyroidectomy, Levo-thyroxine, Ulcer healing.

INTRODUCTION

Retrospective studies of the epidemiology of thyroidectomy from the end of the 20th century till recent reports have shown, a concurrent modest increase in inpatient and pronounced increase in outpatient thyroidectomies, with a consequential demographic shift and staggering economic impact (Sun *et al.*, 2013). Thyroxine regulates metabolism (Muller *et al.*, 2014), protein synthesis (Kenessey and Ojamaa, 2006) and the body's sensitivity to other hormones (Shah, 2012). In the gastrointestinal (GI) tract, thyroid hormone is important for the maturation of intestinal mucosal cells (Bronk and Parsons, 1965), growth of intestinal mucosa crypts, and stimulation of cellular mitosis and growth (Carriere, 1966).

The epidemiology of gastric ulcer disease reflects complex, multifactorial aetiologies. It was first described in 1835, but mortality from the disease has fallen dramatically for birth cohorts since the 20th century (Sonnenberg, 2007). This may not be unconnected with the depth of knowledge garnered within this period in the course of management of gastric ulcers. There is a growing evidence of the presence and participation of reactive oxygen molecules in the aetiology and pathophysiology of clinical conditions and human diseases, including thyroidectomy and gastric ulcer disease (Yoskikawa *et al.*, 1989). Alteration of thyroid function from the euthyroid state (i.e., hyperthyroidism or hypothyroidism) has been implicated in increased generation of superoxide radicals and hydrogen peroxides – free reactive oxygen species reminiscent of oxidative stress (Fernandez and Videla, 1993; Resch *et al.*, 2002; Yilmaz *et al.*, 2003).

Oluwole and Saka, (2007) established the antiulcerogenic ability of the thyroid hormone in indomethacin-induced gastric ulcer model while Olaleye *et al*., (2013) confirmed its healing potential on already formed chronic gastric ulcers using acetic acid. It was opined that the acceleratory effect of thyroid hormone on gastric ulcer healing is achieved by enhanced inflammatory and proliferative phases of healing as well as increased white blood count during the healing process, while thyroidectomy was said to delay these processes (Olaleye *et al.* 2013). Recently, Adeniyi *et al.* (2014) assessed and reported oxidative stress (with lipid peroxidation as marker) and apoptosis (with DNA fragmentation as marker) during healing of acetic acid induced gastric ulcers after thyroidectomy and thyroxine treatment. This (current) study was designed to evaluate/access healing of ischemic-reperfusion induced gastric ulceration in thyroidectomised and thyroxine treated rats with focus on oxidative stress during the inflammatory stage of gastric healing.

Clinical and experimental studies have demonstrated imbalance between free radical and anti-oxidant system in subjects with thyroid dysfunction. There is conflicting report on the effect of thyroxine replacement therapy and subsequent hyperthyroidism on anti-oxidant activity (Ali *et al.* 2005 and Chandra *et al.* 2010) have reported increased Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GTP) activities in hyperthyroid state, but Fernandez *et al.* (1988) reported reduced anti-oxidant activity in hyperthyroid state. Hypothyroidism has also been correlated with decreased antioxidant activity (Pasupathi and Latha, 2008).

Nitric oxide (NO), is a signaling molecule (Hou *et al.*, 1999) synthesized endogenously by its' synthase enzyme (nitric oxide synthase) from L-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate (NADPH) (Stuehr, 2004). Its release reportedly accounts for the biological activity of endothelium-derived relaxing factor – a vasodilatory mediator (Palmer *et al.*, 1987) which is of utmost importance for protecting against ischemic damage in organs (Culotta *et al.*, 1992). Its dilatory effect in the vascular endothelium has been well elucidated (Lowenstein *et al.*, 1994), as well as its effect in limiting platelet activation, adhesion and aggregation with involvement in the inhibition of cyclooxygenase-1 via guanylyl cyclase activation (Loscalzo, 2001).

To our knowledge, there is no information on the role played by NO during ischemia-induced gastric ulcer healing, despite its known role in protecting against ischemic damage in organs. Albeit, this study attempts to compare results from the ischemia-induced gastric ulcer models with previous ulcer models (based on the knowledge of their different mechanism of ulcer formation). It also sought to investigate the probable mechanism(s) involved during the inflammatory stage of gastric ulcer healing with thyroxine treatment.

MATERIALS AND METHODS

Drugs and Kit

Levothyroxine Sodium was purchased from Mercury Pharma (Generics) Ltd., Croydon, UK and Procaine Penicillin was purchased from Guorui Pharmaceutical Co. Ltd., China. Thyroid [(T_3), (T_4), Thyroixine stimulating hormone (TSH)], ELISA kits were purchased from Fortress Diagostics Limited, Antrim, UK.

Experimental Animals

Sixty adult male Wistar rats (150 – 200g) were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan. The animals were assessed for good health, acclimatized for two weeks and maintained under standard laboratory conditions: room temperature of 23-25^{o}C, relative humidity (55%) and 12hr light/12 hour dark cycle. They had free access to standard commercial rat diet and clean tap water *ad libitum*.

The animals were then randomly divided into 4 groups of 5 animals namely: Normal control (NC), Sham operated ulcerated (SU), Thyroidectomised ulcerated untreated (ThU), and Thyroidectomised ulcerated treated with (100µg/kg/day) of Levothyroxine (ThU + T4) for 35 days according to the method of Olaleye and Elegbe (2005).

Measurement of Animal Weight:

Animals in all groups were weighed twice weekly throughout the period of the study using a digital weighing scale (Camry EK8012). The percentage change in weight over the period was calculated using normal control as the reference for l the other groups.

Thyroidectomy:

Thyroidectomy was done using the method reported by Olaleye *et al.*, (2013). Briefly, a midline incision was carefully made in the neck region of the animals under Xylazine (5 mg/kg b.w) and Ketamine (60 mg/kg b.w) anaesthesia. The skin, fascia and muscle covering the thyroid gland were also bilaterally retracted and the thyroid gland carefully and completely excised leaving the parathyroid gland. Care was taken to spare the

recurrent laryngeal nerve. Cotton wool dipped in methylated spirit was used to clean the surgical site to eliminate microbial contamination. The incision was then sutured back, cleaned with methylated spirit, procaine penicillin powder paste and the animals were allowed to awake before returning them to their cages.

Sham Operation:

This was as earlier described by Olaleye *et al.*, (2013). The exposed thyroid gland was left intact after the midline incision was made in the neck region. Cotton wool dipped in methylated spirit was used to clean the surgical site. The incision was then sutured back, cleaned with procaine penicillin powder paste and the animals were allowed to awake before returning them to their cages.

Ulcer Induction:

Thirty five (35) days after thyroidectomy, sham operation and oral thyroxine treatment, gastric ulcers were induced in groups 2, 3 and 4 by the ischemia – reperfusion model of Wada *et al.*, (1996) and Mard *et al*., (2012). The rats were fasted for 24 hours, after which they were anaesthetized using sodium pentobarbital (15mg/kg b.w). Laparatomy was performed to expose the stomach after which the celiac artery was clipped using a bull-dog clip for 30 minutes before reperfusing for another 30 minutes. The incision site was cleaned with methylated spirit and sutured back after the stomach had been carefully tucked back into the visceral. The animals were allowed to awake and returned to their cages having access to standard diet and water *ad libitum*.

Gastric Acid Secretion:

Gastric acid study was carried out according to the method of Ghosh and Schild (1958) with slight modifications. The rats were fasted for 24 hours, after which they were anaesthetized using Sodium Pentobarbital (15mg/kg b.w). Gastric acid was collected via a pyloric cannula at 10 minutes intervals and 10 mL of the stomach perfusate was titrated against 0.0025N Sodium hydroxide (NaOH) solution to determine acidity using 2-3 drops of phenolphthalein as indicator according to a method described by Olowookorun, (1975).

Calculations: Titrable acidity in each sample was calculated using the relationship $C_A = (C_B \times V_B) / V_A$ and expressed in mmol/10mins
Where C_A= Acid concentration of gastric secretion, C_B= Concentration of NaOH, V_A= Volume of gastric effluent, V_B= Volume of NaOH used to titrate. The pH of the effluent was obtained from the equation; $pH = -\log [C_A]$.

Whole blood collection

Whole blood (2 mLs) was collected from retro orbital sinus of rats into 5 mLs EDTA bottles and processed for haematological analysis and thyroxine assay.

Measurement of Plasma Thyroxine (T_4), Triiodotyronine (T_3) and Thyroid Stimulating Hormone (TSH):

After 35 days post-surgery and of drug treatment, plasma T_4, T_3 and TSH was assayed using the Enzyme-Linked Immuno-Sorbent Assay (ELISA) technique with a Fortress diagnostics kit.

Hematological Variables:

Determination of Packed Cell Volume (PCV) was carried out as described by Sorokin, (1973).

Evaluation of Red Blood Cell (RBC) count was as described by the method of Rowan, (1983).

Determination of Hemoglobin Concentration (Hb) was as described using the Sahli's method (van Lerberghe *et al.*, 1983, Gammon and Baker 1977)

Estimation of Total White Blood Cell *(TWBC)* Count was according to the method of Haeney, (1976) while estimation of ***Differential White Blood Cell Count*** was according to the method of Bessman and Feinstein, (1986) using the Leishman's stain.

Platelet Count was determined according to the method of Brecher and Cronkite, (1850).

Ulcer Scoring and tissue harvest:

Degree of ulceration was assessed by macroscopic examination using a 2X magnification hand lens. The stomach was opened along the greater curvature, bathed in normal saline, spread out with pins on a cork board, and then measured. The ulcerated area was calculated according to the method of Kulakarni (1987), using the following criteria: 0 for normal stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcers>3mm<5mm, 3 for ulcers 5mm.

Thereafter, about 50mg portion of the stomach was cut and kept in phosphate buffer saline sample bottles and homogenized with a tissue homogenizer. This was then centrifuged at 4ºC at maximum speed for 30 seconds. The supernatants from each sample were kept in the freezer at -20ºC pending biochemical assays.

Biochemical Analysis

Protein Concentration of gastric samples was according to the method of Gornal *et al*., (1949), with slight modification. Potassium was added to the Biuret reagent to prevent precipitation of CU^{2+} as Cuprous Oxide.

Assessment of Lipid Peroxidation: The degree of Lipid peroxidation was determined according to the method of Varshney and Kale, (1970).

Determination of Superoxide Dismutase (SOD) Activity: The SOD activity in gastric tissue homogenates was determined as described by Misra and Fridovich, (1972).

Determination of Catalase Activity: The Catalase activity of the gastric tissue homogenate was determined according to the method of Claiborne, (1985).

Total Nitrite Assay: This was carried out using the method described by Ignarro *et al*., (1987) based on diazotization reaction that was originally described by Griess, (1879).

Histology:

Briefly, a portion of the stomach samples were fixed in 10% formalin and later embedded in paraffin and sectioned at 5µm in an automated microtome. Staining was done with the Haematoxylin and Eosin technique after which the tissues were examined for inflammation, granulation, regeneration and vascular integrity.

Statistical Analysis:

The Mean ± Standard Error of Mean (S.E.M) of all values were obtained and analysed using one way ANOVA with Bonferorri post hoc test and student's 't' test to compare differences among variables . The p-value <0.05 was considered statistically significant.

RESULTS

Percentage Body Weight Change:

Thyroidectomised thyroxine treated animals showed significant weight loss compared with the control and thyroidectomised untreated groups, P<0.05 (Figure 1).

Plasma Level of Thyroxine, Triiodothyronine and Thyroid Stimulating Hormone:

A significant increase in the plasma triiodothyronine and tetraiodothyronine (T_3 and T_4) levels of the thyroidectomised thyroxine treated ulcerated animals (ThU + T_4) was observed 35 days post-surgery when compared with the thyroidectomised ulcerated untreated animals (ThU). The plasma thyroid stimulating Hormone (TSH) level was significantly decreased in the thyroidectomised thyroxine treated animals compared with the thyroidectomised ulcerated untreated animals (Table 1).

Effect of thyroxine treatment on the basal gastric acid secretion, acidity and pH of ischemic reperfused gastric ulceration 1 hour, and days 3 and 7 post ulceration:

Basal acid output was significantly lower in normal Control, Sham untreated ulcerated thyroidectomised thyroxine treated ulcerated groups compared with the thyroidectomised untreated ulcerated group on days 3 and 7 post ulceration (Table 2).

Effect of thyroxine treatment on the mean ulcer score during ischemia re-perfused gastric ulceration:

There was a significant decrease in the mean ulcer score of the Thyroidectomised thyroxine treated ulcerated group compared with thyroidectomised untreated ulcerated and Sham ulcerated groups compared on all days of experimentation (Table 3)

Effect of thyroxine treatment on some haematological parameters of ischemic-reperfused gastric ulceration 1 hour, and, days 3 and 7 post ulceration:

Thyroidectomised thyroxine treated ulcerated animals had a significantly increased RBC, Hb and PCV by 1 hour and day 3 post ulceration compared with normal, sham and thyroidemised untreated ulcerated groups. There was however no significant changes in the PCV, Hb and RBC amongst all the groups by day 7 post ulceration, (Table 4). A significantly increased platelet count was observed in the thyroidectomised thyroxine treated ulcerated animals by days 3 and 7 post ulceration compared with normal control and thyroidectomised untreated ulcerated groups, (Table 4).

Effect of thyroxine treatment on total and differential white blood cell counts of ischemic reperfused gastric ulceration 1 hour, and, days 3 and 7 post ulceration:

There was a significant increase in the total and differential white blood cell count of the thyroidectomised untreated ulcerated group compared with other experimental groups by 1 hour and day 3 post ulceration. Neutrophil count significantly increased in the thyroidectomised untreated ulcerated group by day 3 while lymphocyte count significantly increased in thyroidectomised thyroxine treated ulcerated group

by day 7 post ulceration. There was no significant difference in monocyte and eosinophil count between the thyroidectomised untreated ulcerated and thyroidectomised thyroxine treated ulcerated groups compared with control groups on all days (1 hour, 3 and 7) post ulceration, Table 5.

Effect of Thyroxine Treatment on Neutrophil / Lymphocyte (N/L) Ratio of Ischemic-Reperfused Gastric Ulceration 1 hour, days 3 and 7 post ulceration:

The neutrophil/lymphocyte (N/L) ratio was significantly reduced in the normal control and thyroidectomised thyroxine treated ulcerated groups compared with the sham and thyroidectomised untreated ulcerated groups by 1 hour, days 3 and 7 post ulceration (Figure 2).

Effect of Thyroxine Treatment on Protein Estimation of Gastric Homogenates During Ischemic Reperfused Gastric Ulceration 1 hour, days 3 and 7 post ulceration:

The normal control and thyroidectomised thyroxine treated ulcerated animals had significantly higher gastric protein level compared with sham and thyroidectomised untreated ulcerated groups by day 7 post ulceration. Protein estimation significantly reduced in sham ulcerated untreated, thyroidectomised untreated ulcerated and thyroxine treated ulcerated groups from 1 hour, days 3 and 7 post ulceration, as there was a gradual and steady decrease in the protein levels of all the experimental groups except the normal animals from 1 hour, days 3 and 7 post ulceration, (Table 6)

Effect of Thyroxine Treatment on Lipid peroxidation/malondialdehyde (MDA) in Ischemic Reperfused Gastric Ulceration 1 hour, and on days 3 and 7 post ulceration:

There was a significant decrease in the malondialdehyde (MDA) level of the normal and thyroidectomised thyroxine treated ulcerated groups compared with the sham and thyroidectomised untreated ulcerated groups on all the days of experimentation (Figure 3).

Effect of Thyroxine Treatment on the Antioxidant Enzymes Activities of Ischemic Reperfused Gastric Ulceration 1 hour, days 3 and 7 post ulceration:

Superoxide dismutase activity was significantly higher in normal control and thyroidectomised thyroxine treated ulcerated animals compared with Sham and Thyroidectomised untreated ulcerated groups by 1 hour, days 3 and 7 post ulceration (Figure 4).

The activity of Catalase was significantly higher in thyroidectomised thyroxine treated ulcerated animals compared with all other experimental groups by 1 hour, days 3 and 7 post ulceration (Figure 5).

Effect of Thyroxine Treatment on Nitirc Oxide (NO) and on Platelet / Nitric Oxide of Ischemic Reperfused Gastric Ulceration 1 hour, days 3 and 7 post ulceration:

Gastric mucosa nitric oxide levels were significantly higher in normal control and thyroidectomised thyroxine treated ulcerated groups than in sham and thyroidectomised untreated ulcerated groups by 1 hour, days 3 and 7 post ulceration (Figure 6). There was a gradual decrease in the nitric oxide (NO) levels of the thyroidectomised thyroxine treated ulcerated animals by 1 hour, days 3 and 7 post ulceration compared with other groups.

The thyroidectomised untreated ulcerated group had significantly higher platelet/nitric oxide ratio compared with the normal control, sham operated and thyroidectomised thyroxine treated ulcerated groups (Figure 7)

Table 1: Plasma thyroxine, triiodothyronine and thyroid stimulating hormones after treatments

Groups	FT_3 (pmol/L)	FT_4 (pmol/L)	TSH (miu/L)
NC	2.6±0.14	14±0.71	<0.01
SU	2.4±0.21	13±0.45	<0.01
ThU	1.5±0.19[a,b]	7.7±0.66[a,b]	1.1±0.03
ThU+T_4	3.1±.0.031[c]	21±0.31[a,b,c]	<0.01

Values are expressed as Mean ± SEM and are considered statistically significant when p value < 0.05. Key of Significance: [a]significant compared with animals in control group (NC), [b]significant compared with animals in control group (SU), [c]significant compared with animals in thyroidectomised group (ThU).

Table 2: Effect of Thyroxine treatment on the Basal Gastric Acid secretion, Acidity and pH of ischemic reperfused gastric ulceration 1 hour, days 3 and 7 post ulceration.

Groups	Basal Acid Output (ml/10mins)			Acidity ($\times 10^{-5}$mmol)			pH		
	Day0	Day3	Day7	Day0	Day3	Day7	Day0	Day3	Day7
NC	0.33±0.02	0.34±0.02	0.23±0.02	8.0±0.01	1.0±0.08	0.57±0.03	4.08±0.04	4.06± 0.04	4.25±0.03
SU	0.33±0.02	0.35±0.02	0.27±0.01	9.15±0.49	9.75±0.00	7.27±0.23[a]	4.06±0.02	4.04±0.00	4.14±0.02[a]
ThU	0.36±0.13	0.48±0.20	0.40±0.00	9.53±0.23	13.0±0.00[a,b]	10.0±0.00[ab]	4.02±0.01	3.89±0.00[a,b]	4.00±0.00[a]
ThU+T_4	0.34±0.01	0.42±0.02	0.23±0.01	8.3±0.00[c]	10.5±0.00[a,c]	6.3±0.00[b,c]	4.08±0.00[c]	3.96±0.00[a,b,c]	4.20±0.00[ac]

Values are expressed as Mean ± SEM and are considered statistically significant when p value = 0.05. Key of Significance: [a]compared with control group (NC), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

Table 3: Effect of thyroxine treatment on the mean ulcer score of ischemia reperfused gastric ulcer 1 hour, days 3 and 7 post ulceration

U - Ulcer, H- Haemorrahge, R - Reddish colouration, S - spot ulcers. Black arrows represent ulcer

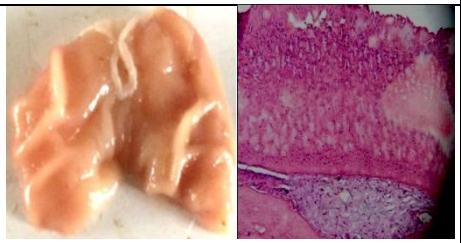
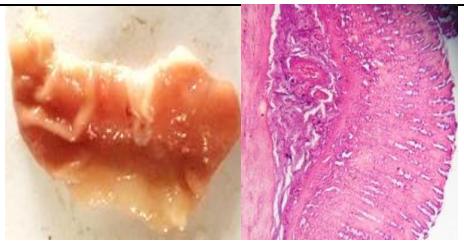
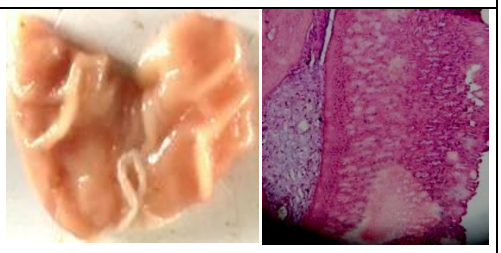
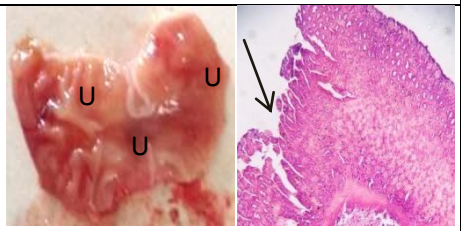
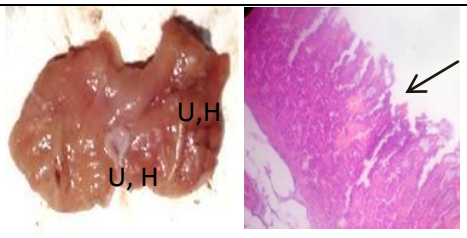
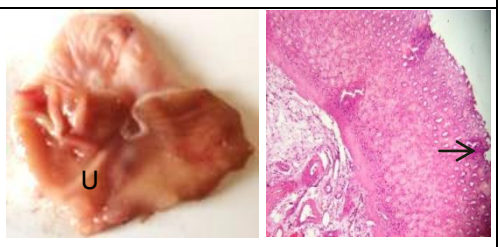
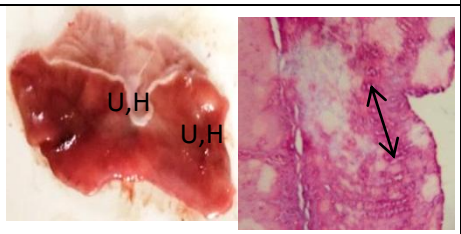
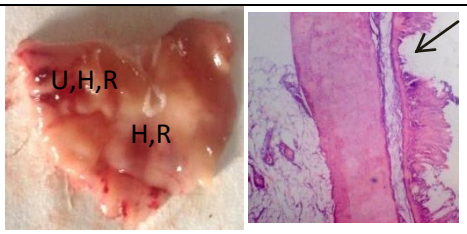
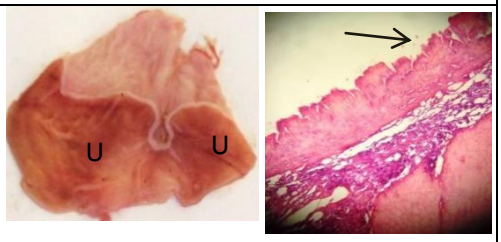
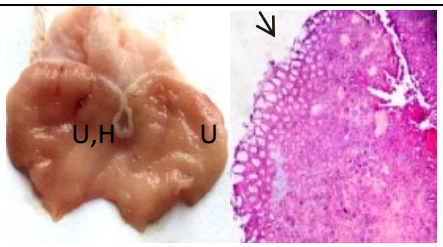
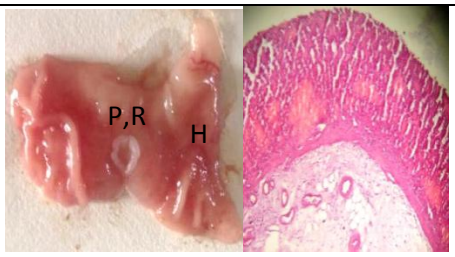
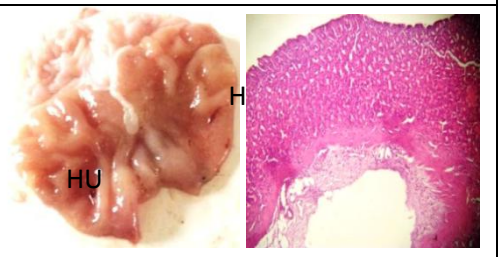
Groups	Day 0 (1 hour)	Day3	Day7
N	 0±0.00	 0±0.00	 0±0.00
SU	 27.8±0.60[a]	 18.5±0.80[a]	 7.0±0.70[a]
ThU	 39.8±4.70[a,b]	 29.5±3.00[a,b]	 15.1±0.70[a,b]
ThU+T4	 16.1±0.30[a,b,c]	 12.0±1.00[a,b,c]	 2.5±1.10[b,c]

Table 4: Effect of Thyroxine treatment on the Packed Cell Volume, Red Blood Cells, Hemoglobin and Platelet Counts of ischemic reperfused gastric ulceration.

Groups	PCV (%)			RBC(millions/cu mm)			Hb (g/dL)			Platelet (millions / cu mm)		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
NC	39.67 ± 0.33	51.33 ± 3.53	42.33 ± 1.45	6.46 ± 0.02	8.33 ± 0.46	7.02 ± 0.34	13.50 ± 0.06	17.77 ± 1.17	14.50 ± 0.59	173,333 ± 33,410	95,330 ± 24,390	89,670 ± 33,410
SU	41.00 ± 1.0	36.6 ± 1.20	44.00 ± 0.58	6.99 ± 0.29	6.54 ± 0.21	7.42 ± 0.12^{a}	14.10 ± 0.35	13.37 ± 0.28	7.42 ± 0.12	177,667 ± 28,260	132,000 ± 17,436	95,330 ± 7,965
ThU	41.67 ± 0.67	32.6 ± 1.45^{a}	44.33 ± 0.67	6.60 ± 0.13	6.23 ± 0.35	7.36 ± 0.05^{a}	13.63 ± 0.12	12.20 ± 0.76	13.0 ± 0.76^{b}	201,700 ± 9667	108,670 ± 11,330	118,000 ± 8000
ThU +T_4	47.33 ± $1.20^{a,b,c}$	47.3 ± $1.20^{a,b,c}$	47.33 ± 1.86^{a}	7.52 ± $0.04^{a,c}$	7.36 ± 0.08^{c}	7.51 ± 0.07^{a}	15.57 ± $0.07^{a,b,c}$	15.10 ± 0.32^{c}	15.00 ± $0.30^{b,c}$	236,700 ± 33,330	196,667 ± $22,930^{a,c}$	202,000 ± $4,000^{a,b,c}$

Values are expressed as Mean ± SEM and are considered statistically significant when p value = 0.05.Key of Significance: [a]compared with control group (NC),[b]compared with control group (SU), [c]compared withthyroidectomised group (ThU).

Table 5:Effect of Thyroxine treatment on White Blood Cell, Neutrophil, Lymphocyte, Monocyte and Eosinophil Counts of ischemic reperfused gastric ulceration

Groups	WBC (millions / cu mm).			Neutrophil(10^3/ µL)			Lymphocyte(10^3/ µL)			Monocyte(10^3/ µL)			Eosinophil (10^3/ µL)		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
NC	7,733 ± 483.33	2,400 ± 76.38	5,016 ± 216.67	29.00 ± 1.53	32.33 ± 3.67	29.00 ± 1.53	72.00 ± 2.52	64.00 ± 4.00	69.33 ± 4.81	1.33 ± 0.33	2.67 ± 0.33	1.33 ± 0.33	1.33 ± 0.33	1.67 ± 0.33	2.67 ± 0.33
SU	12,100 ± 50[a]	12,533 ± 133.33[a]	5,800 ± 351.19[a]	28.33 ± 1.76	32.33 ± 1.33	31.67 ± 0.88	76.00 ± 3.22	66.33 ± 0.88	66.33 ± 1.20	3.00 ± 0.58	2.67 ± 0.33	3.00 ± 0.00[a]	2.00 ± 0.57	2.33± 0.33	3.00 ± 0.57
ThU	10,333 ± 166.67[a,b]	11,700 ± 556.78[a]	10,500 ± 250[a,b]	21.33 ± 2.03	22.33 ± 0.88[a, b]	25.67 ± 1.86	82.67 ± 3.71	74.00 ± 1.73	72.33 ± 1.45	4.33 ± 0.88	2.67 ± 0.33	2.33 ± 0.33	2.33 ± 0.33	2.33 ± 0.67	4.00 ± 1.00
ThU + T_4	14,033 ± 16.67[a,b c]	18,467 ± 233.33[a,b, c]	6716 ± 783.33[c]	27.33 ± 2.60	32.67 ± 1.45[c]	40.33 ± 2.96[a, c]	67.33 ± 3.84	64.00 ± 2.31[c]	58.67 ± 1.45[a, c]	3.00 ± 0.58	1.33 ± 0.33	2.33 ± 0.90	0.67 ± 0.33	1.33 ± 0.33	2.00 ± 0.57

Values are expressed as Mean ± SEM and are considered statistically significant when p value = 0.05.Key of Significance: [a]compared with control group (NC), [b] compared with control group (SU), [c]compared with thyroidectomised group (ThU).

Table 6: Effects of thyroxine treatment on protein estimates of gastric homogenates during ischemic reperfused gastric ulceration

Groups	Protein (mg/mL)		
	Day0	**Day3**	**Day7**
NC	0.837±0.03	0.84±0.03	0.82±0.01
SU	1.278±0.04[a]	0.623±0.07	0.563±0.05[a]
ThU	0.980±0.03[a,b]	0.743±0.03	0.673±0.01[b]
ThU+T4	1.067±0.02[a,b]	1.017±0.07[b,c]	0.707±0.04[c]

Values are expressed as Mean ±SEM and significant when p<0.05. Key of Significance: [a]compared with control group (NC), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

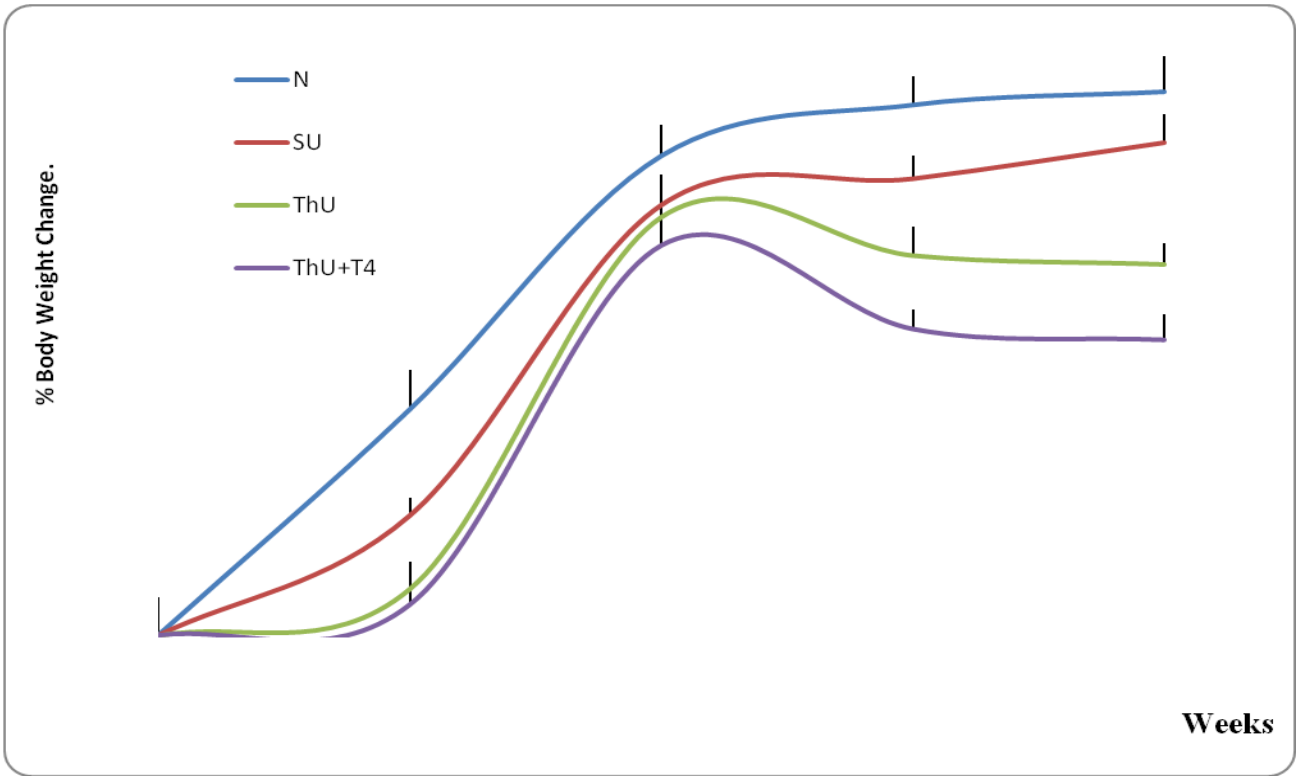


Figure 1: Effect of thyroxine treatment on percentage body weight change during ischemia re-perfused gastric ulceration.

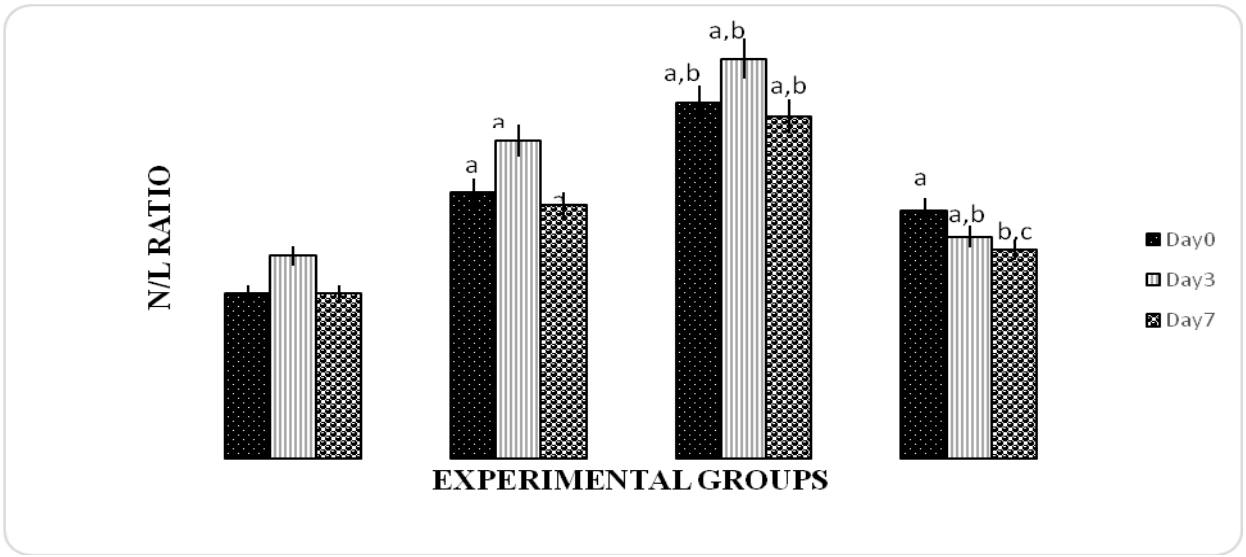


Figure 2: Effect of Thyroxine treatment on the Neutrophil/Lymohocyte (N/L) ratio of ischemic reperfused gastric ulceration.

Values are expressed as Mean ±SEM and significant when p<0.05. Key of Significance: [a]compared with control group (N), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

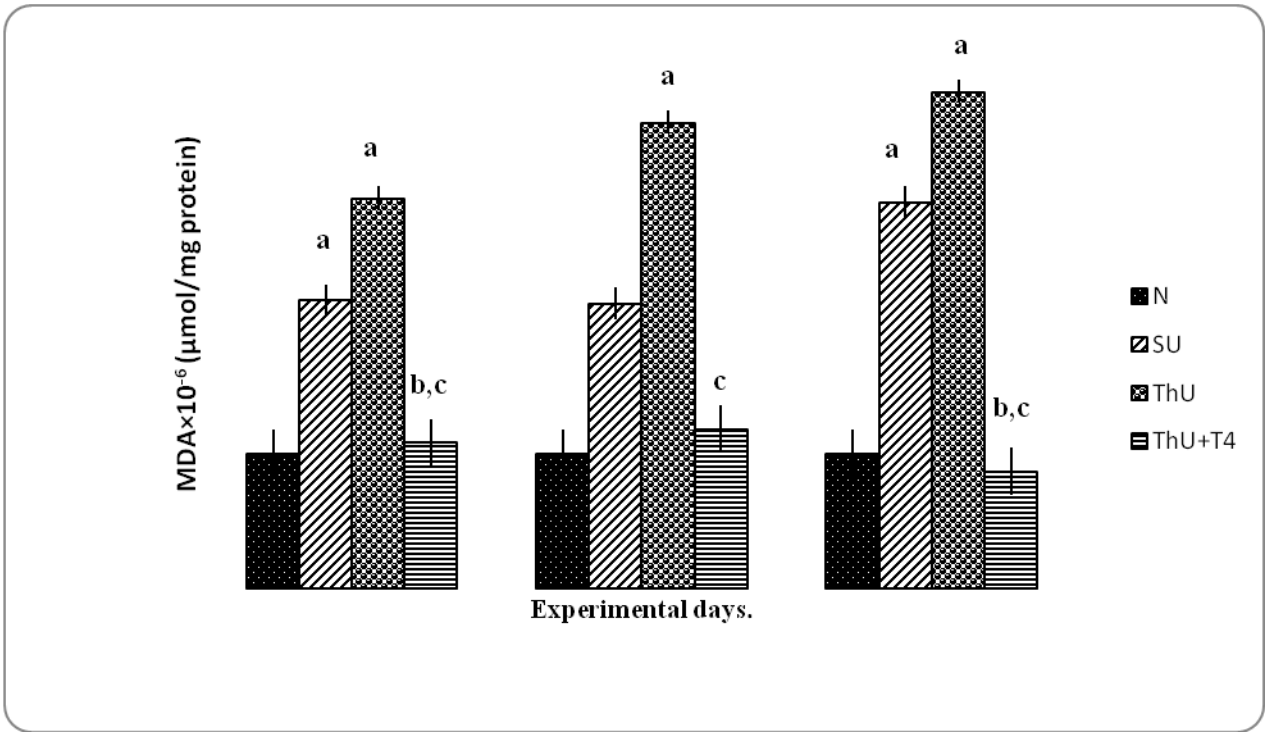


Figure 3: Effect of Thyroxine treatment on the Lipid peroxidation (MDA) of ischemic reperfused gastric ulceration:

Values are expressed as Mean ±SEM and significant when p<0.05. Key of Significance: [a]compared with control group (N), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

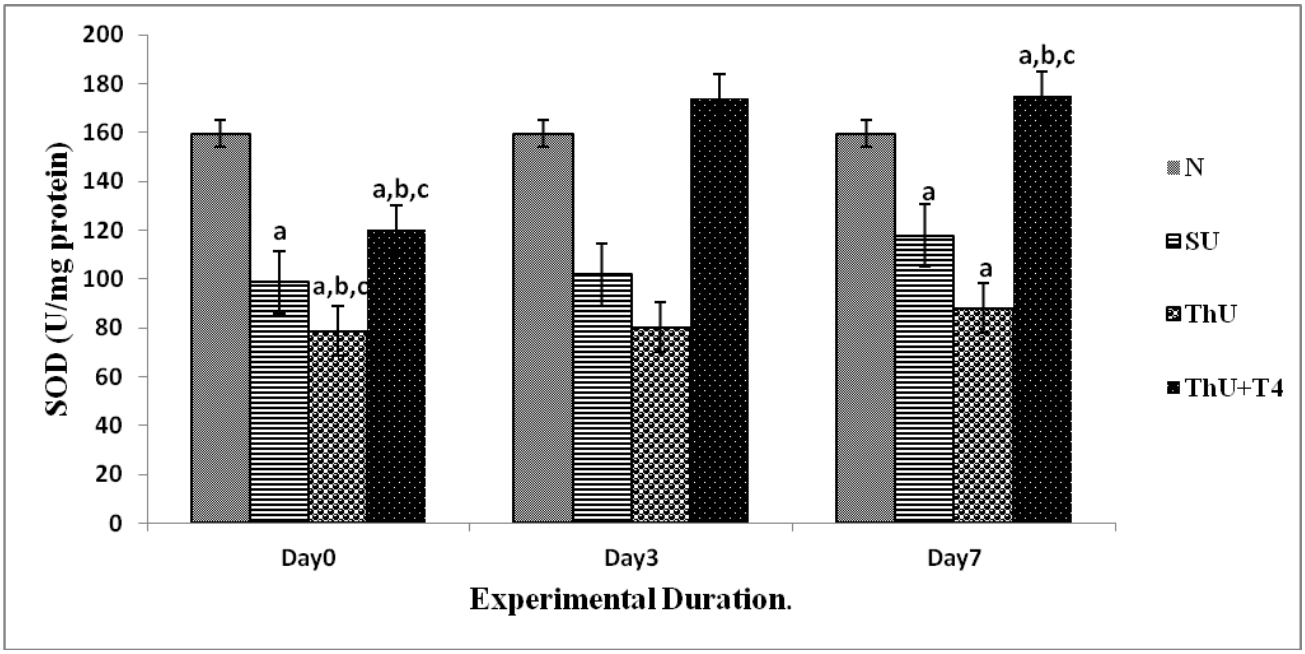


Figure 4: Effect of Thyroxine treatment on Superoxide Dismutase Level of gastric homogenates during ischemic reperfused gastric ulceration

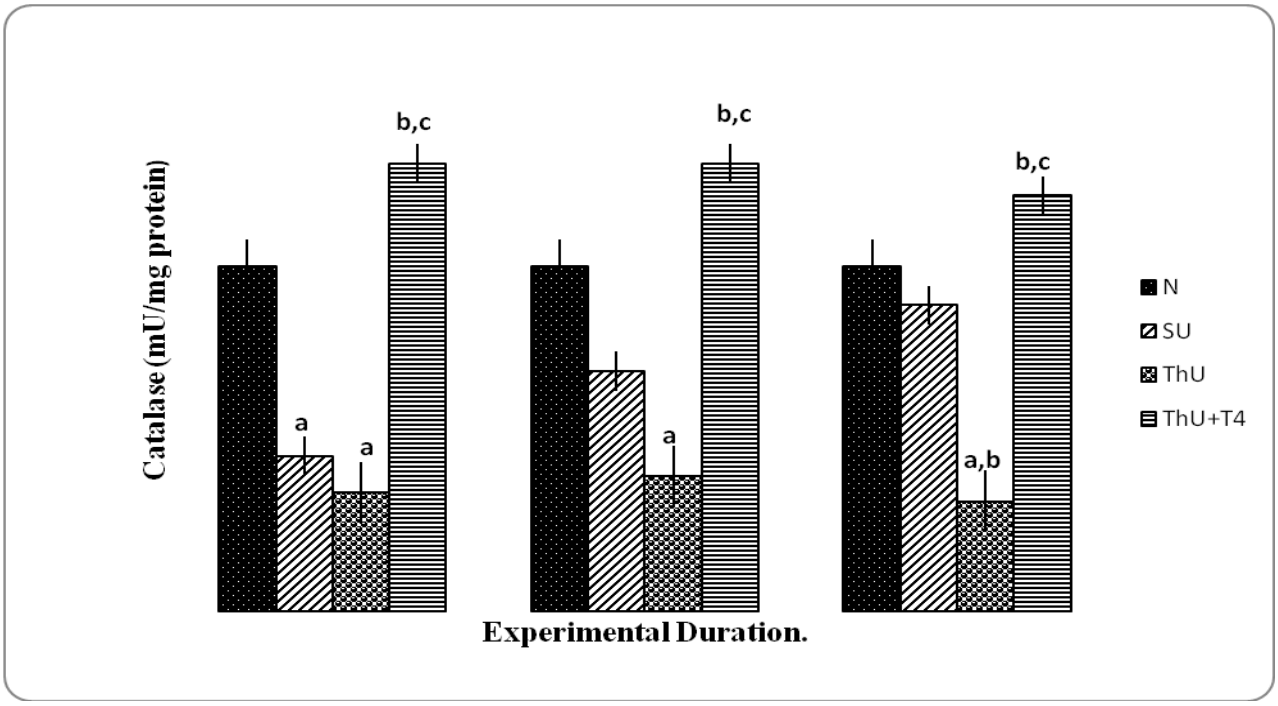


Figure 5: Effect of Thyroxine treatment on Catalase level of ischemic reperfused gastric ulceration

Values are expressed as Mean ±SEM and significant when p<0.05. Key of Significance: [a]compared with control group (N), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

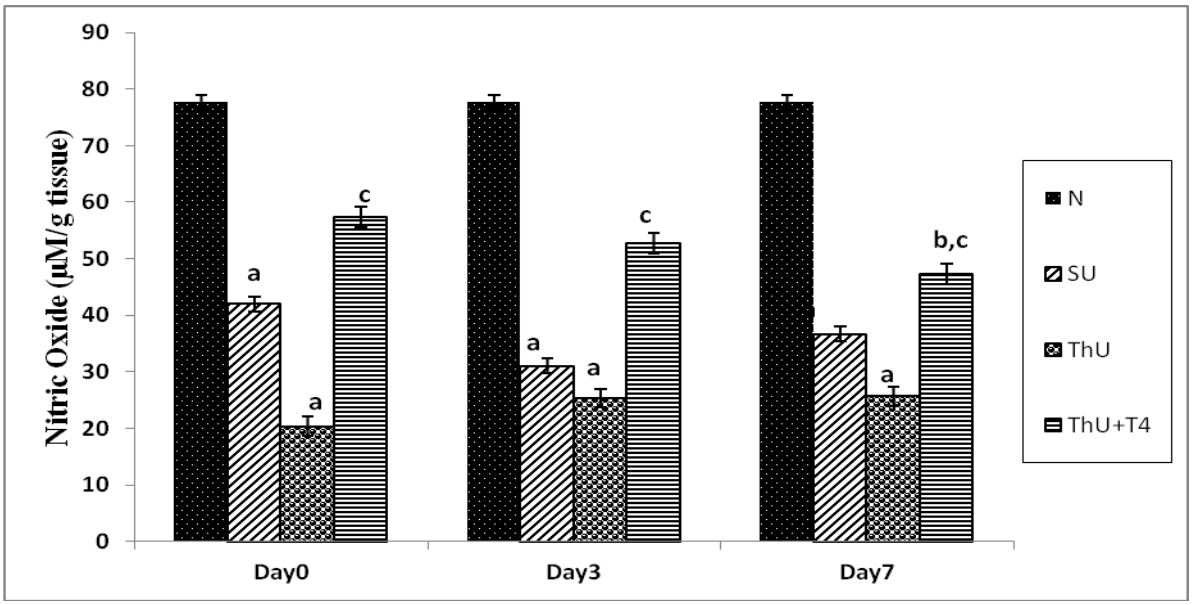


Figure 6: Effect of Thyroxine treatment on the Nitric oxide Level of ischemic reperfused gastric ulceration

Cortical bars represents Mean ±SEM. Values are significant when p=0.05. Key of Significance: [a]compared with control group (N), [b]compared with control group (SU), [c]compared with thyroidectomised group (ThU).

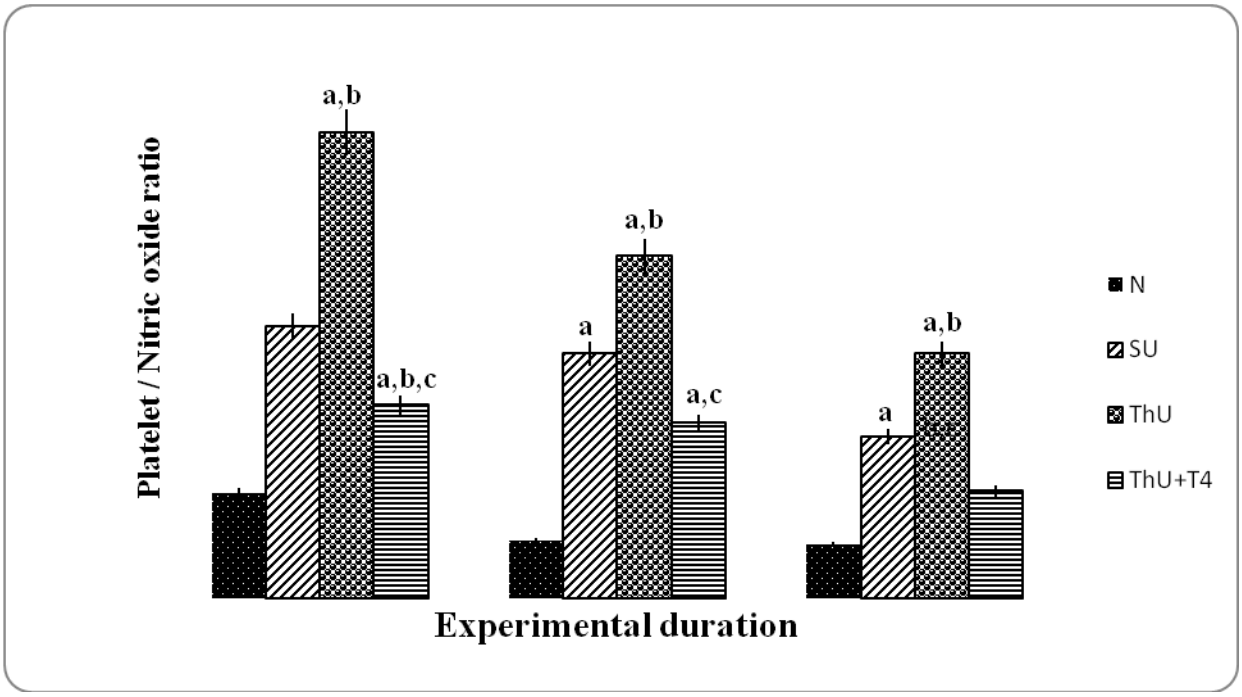


Figure 7: Effect of Thyroxine treatment on the Platelet / Nitirc oxide ratio of ischemic reperfused gastric ulceration

DISCUSSION

Ischemia-Reperfused induced gastric ulcer in humans is mostly as a consequence of compression of the celiac artery mostly by the median arcuate ligament (Dunbar *et al*., 1965, Mard *et al.,* 2012). The pathophysiology may be related to both ischemic and neuropathic mechanisms but symptoms appear to be relieved following surgical decompression of the celiac artery (Jimenez *et al*., 2012). Oxidative stress has also been implicated in the build-up of ulcerations post gastric ischemic injury (Cui *et al.,* 2013); Kim and Kim (2007) also reported a case of celiac artery thrombosis, and splenic infarction which was surgically treated.

Weight gain has been delineated as a clinical indicator of hypothyroid status (Annadanam *et al.,* 1998). Results from this study shows that hypothyroid animals had appreciable weight gain than thyroidectomised thyroxine treated animals. This is in line with earlier reports that hypothyroidism causes a resultant hypometabolism, hypercholesterolemia as well as increased body weight (Baron, 1956, Martin *et al.,* 1985, Forehead *et al.,* 2000).

Thyroid hormone has been documented to accelerate healing in the gastric mucosa (Olaleye *et al.,* 2013). Meanwhile, Guo *et al.,* (2002) have shown that increased blood flow is important in supplying oxygen and nutrients to a healing mucosa. Thyroidectomy (in this study) might have caused diminished delivery of blood to gastric mucosa coupled with the resulting ulceration of gastric mucosa due to occlusion of celiac artery (ischemia-reperfusion model of ulcer induction). This probably led to formation of new breaches in the gastric mucosa hence reducing healing rate of formed ulcers. Treatment with Thyroxine (in thyroidectomised animals) had an inverse effect by promoting vascularisation and blood supply to the ulcerated gastric mucosa, leading to an increased availability of oxygen and nutrients to the gastric mucosa cells involved in healing. Thyroxine treatment in thyroidetomised animals grossly reduced mean ulcer scores hence increased healing rate compared with thyroidectomised untreated and sham operated animals.

This accelerated ulcer healing might have been enhanced by the appreciable reduction in the basal gastric acid secretion in thyrodectomised L-thyroxine treated rats compared with thyroidectomised untreated rats, 1 hour, 3 and 7 days post ulcer induction. This further corroborates the findings of Nasset and Goldsmith, (1961) and Rafsanjani *et al*., (2003). They observed that crystalline thyroid hormones, including L-thyroxine, produced less striking depression of acid secretion; an observation they confirmed by studies on rats and they suggested that the thyroid gland might produce a non-thyroxine factor which influenced the stomach. [Goldsmith and Nasset, (1959), Wiersinga and Touber (1980)].

Thyroxine treatment in thyroidectomised animals have been reported to enhance RBC, Hb and PCV blood variables, (Olaleye *et al*., 2013, Osonuga *et al*., 2014) similar results were found in the course of this study between 1 hour and day 3 post ulceration. The increased

red blood cell and hemoglobin count in the thyroxine treated animals probably increased the oxygen and nutrient to the ulcerated site in the thyroidectomised thyroxine treated animals, thereby enhancing healing of induced gastric ulcers.

Increased mean platelet volume has also been reported in hyperthyroid patients (Ford *et al.,* 2006) as thyroid function has been implicated on having a toll on coagulation factors (Debeij *et al.,* 2010). Hypothyroidism causes decreased synthesis of clotting factors (Hoefbauer and Heufelder, 1997; Squizzato *et al.,* 2007) and decreased response to adrenergic stimulation (Myrup *et al.,* 1995; Franchini *et al.,* 2010). Results on platelet count in the thyroidectomised untreated and thyroixine treated ulcerated groups obtained in this study are in line with these earlier reports.

Varying levels of immunosuppression have been reported in animals (Olaleye *et al.,* 2013) with hypothyroidism which was reversible upon treatment with thyroxine. This study found white blood cell count to be elevated in thyroxine treated animals than in control and thyroidectomised untreated groups on 1 hour and day 3 post ulceration. This may explain another probable mechanism of accelerated healing of ulcer in the thyroxine treated group (Haffor, 2010). The neutrophil/lymphocyte ratio (NLR) a marker of systemic inflammatory response was found to be elevated in the thyroidectomised untreated animals than control and thyroxine treated groups. Results obtained from this study were in line with Kim *et al.,* (2013) observations as they found elevated NLR in patients with thyroid nodules than in those without it.

Persike *et al.,* (1948) as well as Gelfand *et al.,* (1985) reported a case of increased protein catabolism in thyroidectomy. This study found increased protein concentration in thyroxine treated animals compared with thyroidectomised untreated animals on days 3 and 7 post ulceration, suggesting the influence of thyroxine in protein anabolism. Present findings also showed elevated in lipid peroxidation of the thyroidectomised untreated animals compared with the control and thyroxine treated groups. This supports the concepts of researchers that have observed high levels of serum malonyldialdehyde in hypothyroid patients (Murat *et al.,* 2014). Hypothyroidism sequel to thyroidectomy may therefore increase malondialdehyde production from the peroxidation of gastric fatty acid membranes.

Results of this research strongly confirmed that thyroidectomy amply reduces Superoxide Dismutase and Catalase levels in gastric homogenates which were ameliorated with thyroxine treatment. Investigators have earlier reported that thyroxine treatment increases the activities of Superoxide dismutase and Catalase (Pereira *et al.,* 1994) in diseased state. This increased antioxidant profile after thyroxine administration confers protection and gastric mucosa defence against free reactive oxygen species.

NO plays a beneficial role in wound healing majorly due to its functional influences on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodelling (Childress *et al.,* 2002; Luo *et al.,* 2005). Recent findings in this study showed that thyroidectomy declined gastric mucosa NO levels while thyroxine replacement therapy caused elevation of this marker of wound healing during the inflammatory stage.

The reduced platelet/NO levels in the thyroidectomised thyroxine treated ulcerated group further corroborates the quick response to injury (ulceration) caused by clamping of the celiac artery. This invariably might have led to the release of some chemo attractants from the platelets adhering to the injury site thus initiating the inflammatory stage of healing. These cascades of events at the wound site modulated by NO acting as chemo attractant for neutrophils and monocytes and probably production of tumor necrotic factor - α (Wahl *et al.,* 1989) in the thyroxine treated groups as seen in this study. Researchers have implicated NO in the regulation of cellular activities of the inflammatory and proliferative phase of healing (Stallmeyer *et al.,* 1999) and this may explain the increased rate of ulcer healing in the thyroxine treated animals.

CONCLUSION

Findings from this study showed that thyroxine treatment facilitates healing of ischemia induced gastric ulcers as a result of the modulatory activities by the increased nitric oxide levels in the regulation of cellular activities during the inflammatory phase of healing.

REFERENCES

Adeniyi O.S, Emikpe B.O, and Olaleye S.B. (2014). Gastric Mucosa Re-epithelisation, Oxidative Stress and Apoptosis during Healing of Acetic Acid-Induced Ulcerations in Thyroixine Treatment and Thyroidectomy on Rats. J. Afr. Ass. Physiol. Sci. 2 (1): 57-67.

Ali Rizvi SA, Husain M, Khan S, Mohsin M 2005. A comparative study of fine needle aspiration cytology versus non-aspiration technique in thyroid lesions. Surgeon, vol. 4, pp. 273-276

Annadanam S.G, Smith M and Vinjamuri S. (1998). Hypothyroidism and Weight gain. Nuclear Medicine Communication; 19(11): 1100

Baron D.N. Hypothyroidism;(1956) Its etiology and relation to hypermetabolism, hypercholesterolemia, and increase in body weight. The Lancet.; 268(6937): 277-281.

Bessman J.D, Feinstein D.I. (1986): Hematology: Clinical Principal and Applications. Blood. 68:506-513.

Brecher G, Cronkite E.P. (1850): Morphology and enumeration of human blood platelets. J Appl Physiol. 3(6): 365-77.

Bronk J.R and Parsons D.S. (1965): Influence of the thyroid gland on the accumulation of sugars in rat intestinal mucosa during absorption. J. Physiol., 179: 323-32.

Carriere R.M. (1966): The influence of thyroid and testicular hormones on epithelium of crypts of liberkuhn in rats intestine. Anat. Rec. 156: 423-31.

Cetinkaya A, Kurutas E.B, Buyukbese M.A, Kantarceken B, Bulbuloglu E. (2005). Levels of Malondiadehyde and Superoxide Dismutase in Subclinical Hyperthyroidism. Mediators of Inflammation. 2005(1): 57-59.

Chandra, R. ; Chatlod, L. R. ; Kumar, S. ; Toppo, S. ; Haque, N. ; Rahman, H.,(2012). Nutritional evaluation of NB-21 hybrid Napier grass for goats. Indian J. Small Rum., 18 (2): 261-263

Childress B.B and Stechmiller J.K. (2002). Role of nitric oxide in wound healing. Biol Res Nurs.; 4(1): 5-15.

Claiborne A: Catalase activity.(1985) In: Handbook of Methods for Oxygene Radical Research. GREENWALD RA (ed), CRC Press, Boca Raton, FL, pp 283-284.

Cui J, Liu L, Zou J, Qiao W, Liu H, Qi Y, Yan C. (2013): Protective effect of endogenous hydrogen sulfide against oxidative stress in gastric ischemia-reperfusion injury. Experimental and Therapeutic Medicine. 5(3): 689-94.

Culotta E. (1992): NO news is good news. Science. 258(5090): 1862-1865.

Debeij J, Suzanne C, van Zaane B, Smit W.A, Corssmit P.M, Rosendaal F.R, Romijn J.A and Dekkers O.M. (2010). The effect of changes in thyroxine and thyroid stimulating hormone levels on the coagulating system. Journal of Thrombosis and Hemostasis. 8: 2823-2826.

Dunbar J.D, Molnar W, Beman F.F, Marable S.A. (1965): Compression of the celiac trunk and abdominal angina. Am J Roentgenol Radium Ther Nucl Med; 95:731.

Edson J.R, Dennis F and Richard P.D. (1975): Low platelet adhesiveness and other hemostatic abnormalities in Hypothyroidism. Annals of Internal Medicine. 82: 3.

Fernandez V. and Videla L.A. (1993): Influence of hyperthyroidism on superoxide radical and hydrogen peroxide production by rat liver submitochondrial particles. Free Radic. Res. Commun. 18, 329– 335.

Fernandez V., Llesuy S., Solari L., Kipreos K., Videla L.A., Boveris A. (1988): Chemiluminescence and respiratory responses related to thyroid hormone-induced liver oxidative stress. Free Radic. Res. Commun.5, 77– 84.

Franchini M, Lippi G, Manazato F, Vescovi P.P and Tagher G. (2010): Hemostatic abnormalities in endocrine and merabolic disorders. European Journal of Endocrinology. 162: 439-451.

Forhead A.J., Li J., Saunders J.C., Dauncey M.J., Gilmour R.S. and Fowden A.L. (2000): Control of ovine hepatic growth hormone receptor and insulin-like growth factor I by thyroid hormones in utero. American Journal of Physiology - Endocrinology and Metabolism Vol. 278 no. 6, E1166-E1174 DOI

Ford, H.C., R.J. Toomath, J.M. Carter, J.W. Delahunt, and J.N. Fagerstrom. (2006): Mean platelet volume is increased in hyperthyroidism. American Journal of Hematology 27: 190–193.

Gammon A and Baker SJ. (1977): Studies in methods of haemoglobin estimation suitable for use in public health programmes. Ind J Med Res; 65:150-156.

Gelfand RA, Hutchinson-Williams KA, Bonde AA, Castellino P, Sherwin RS. (1987):

Catabolic effects of thyroid hormone excess: the contribution of adrenergic activity to hypermetabolism and protein breakdown. Metabolism.;36(6):562-9.

Ghosh M.N and Schild H.O. (1958): Continuous Acid Secretion in the rat. Pharmacol;13:54-61.

Goldsmith, D.P.J., and Nasset, E.S. (1959): Relation of thyroid togastric acid secretion in the anaesthetized rat. Amer. J. Physiol.,197, 1-4.

Gornal AG, Bardawill CJ, David MM (1949): Determination of serum protein by means of Biuret reaction. J. Biol. Chem. 177: 571-576.

Green M.F., and Kunde M.M. (1930): Acidity of gastric contents of normal, cretin and hyperthyroid rabbits. Ibid., 95, 626-629.

Griess, P. (1879): Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt "Ueber einige azoverbindungen." Chem. Ber.12, 426–8.

Guo, Y., Markus, M., and Demissie, M. (2002): Uncertainty of nitrate-N load computation for agricultural watersheds. *Water Resources Research*. DOI: 10.1029/2001WR001149

Haeney M.R. (1976): Erroneous values for the total white cell count and ESR in patients with cryglobulinaemia. J Clin Pathol. 29(10): 894-97.

Haffor A.S (2010): Effect of myrrh (*Commiphora molmol*) on leukocyte levels before and during healing from gastric ulcer or skin injury. J Immunitoxicol; 7(1): 68-75.

Hoefbauer L.C and Heufelder A.E. (1997): Coagulation disorders in thyroid diseases. European Journal of Endocrinology. 136: 1-7.

Hou Y.C, Janczuk A, Wang P.G. (1999): Current trends in the development of nitric oxide donors. Current pharmaceutical design. 5(6): 417-41.

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987): Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A. 84(24):9265-9

Jimenez J.C, Harlander-Locke M, Dutson E.P. (2012): Open and laparoscopic treatment of median arcuate ligament syndrome. J Vasc Surg; 56:869.

Kenessey A, and Ojamaa K. (2006): Thyroid hormone stimulates protein synthesis in the cardiomyocyte by activating the Akt-Mtor and p70S6K pathways. J Biol Chem; 281(30): 20666-72.

Kim C.W and Kim J.W, (2007): "Celiac artery thrombosis and splenic infarction in a patient with protein s deficiency," The Korean Journal of Gastroenterology, vol. 49, no. 6, pp. 390–394.

Kim J, Park T, Jeong S, Jeong C, Ju Y, Lee Y, Hong S, Ha W, Choi S, Jung J. (2013): Prognostic importance of baseline neutrophil to lymphocyte ratio in patients with advanced papillary thyroid carcinomas. Endocrine. 46(3): 526-531.

Kulakarni S.K. (1987): Handbook of Experimental pharmacology. Vallabh Prakash. 148-150.

Loscalzo J. (2001): Nitric oxide insufficiency, platelet activation, and arterial thrombosis. Circulation research. 88: 756-762.

Lowenstein C.J, Dinerman J.L and Snyder S.H. (1994): Nitric oxide: A physiology messenger. Ann Intern Med. 120(9): 227-37.

Luo J.D and Chen A.F. (2005): Nitric oxide: a newly discovered function on wound healing. Acta Pharmacol Sin.; 26(3): 259-264.

Mard S.A, Neisi N, Darbor M., Hassanpour M., Makvandi M and Solgi G. (2012): β-carotene protects the gastric mucosa against ischemia-reperfusion injury in rats. Eur cytokine Netw. 23 (4): 173-8.

Martin D. Epelbaum J. Bluet-Pajot M.T. Prelot M. Kordon C. and Durand D. 1985): Thyroidectomy Abolishes Pulsatile Growth Hormone Secretion without Affecting Hypothalamic Somatostatin. Neuroendocrinology; 41: 476–481.

Misra H.P and Fridovich I. (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem.;247(10):3170–3175

Muller R, Liu Y.Y, Brent G.A. (2014): Thyroid hormone regulation of metabolism. Physiol Rev. 94(2): 355-82.

Murat K, Murat A, Ayse A, Halit D, Mehmet F. (2014): Oxidative stress in patients with thyroidectomy and thyroparathyroidectomy under replacement therapy.; 48(1): 1007.

Myrup B, Bregengard C and Faber J. (1995): Primary Hemostasis in thyroid disease. Journal of Internal Medicine. 238: 59-63.

Nasset E. Sand Goldsmith D. P. J. (1961): Effect of thyroid on gastric secretion and metabolism. Ibid., 201, 567-570.

Ogihara Y, Okabe S. (1993): Effect and mechanism of Sucralfate on healing of acetic acid induced gastric ulcers in rats. J Physiol Pharmacol; 44: 109-18.

Olaleye S.B, Adeniyi O.S, Emikpe B.O. (2013): Thyroxine Accelerates Healing Of Acetic Acid Induced Gastric Ulcer In Rats. Archives of Basic and Applied Medicine. 1 (1): 76-85.

Olaleye, S. B. and Elegbe,R.A. (2005): Acid secretion in isolated and intact stomach of thyroidectomised and thyroxine-treated mice. The Tropical Journal of Health Sciences Volume 12 (2): 35 – 39

Olowookorun M.O. (1975): Influence of sex hormones and Atropine Sulphate on gastric acid secretion in the rat. J.N.G. Vet. Med. Assoc. 4(2): 72-74

Oluwole S.O and Saka M.T. (2007): Effect of thyroid hormone on gastric mucous secretion around indomethacin-induced gastric ulcers in rats. J Med Sci 7(4): 678-681.

Osonuga I.O, Olowookorun M.O., Iquot I.S, and Akinola B.O. (2014): Influence of Thyroxine on Blood Parameters and Liver Enzymes in Adult Male and Female Rats. Afr. J. Biomed. Res. Vol.17, 53- 56.

Palmer R.M, Ferrige A.G and Moncada S. (1987): Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524-526.

Pasupathi P. and Latha R. (2008): Free Radical Activity and Antioxidant Defense Mechanisms in Patients with Hypothyroidism. Thyroid Science 3(12):CLS1-6.

Pereira B, Costa Rosa L.F.B.P, Safi D A, Bechara E J H, and Curi R (1994): Control of superoxide dismutase, catalase and glutathione peroxidase activities in rat lymphoid organs by thyroid hormones J Endocrinol 140 (1) 73-77.

Persike E.C. (1948): Increased protein Catabolism in thyroidectomised rats; rates of urine urea excretion and serum urea concentrations. Endocrinology.; 42(5): 356-362.

Rafsanjani FN, Z Asl S, Naseri MK, Vahedian J. (2003): Effects of thyroid hormones on basal and stimulated gastric acid secretion due to histamine, carbachol and pentagastrin in rats. Saudi Med J. 24(4):341-6.

Resch U., Helsel G., Tatzber F., and Sinzinger H. (2002): Antioxidant status in thyroid disfunction. Clin. Chem. Lab. Med., 40:1132-1134.

Rowan R.M. (1983): Blood cell volume analysis-A new screening technology for the hematologist. Albert Clerk and Compant Ltd, London.

Shah S.B. (2012): Allergy – Hormone Links. Jaypee Brothers Medical Publishers Ltd. First Edition. 978-93-5025-013-6.

Sorokin C. (1973): Dry weight, packed cell volume and optical density. Handbook of phycological methods: Culture methods and growth measurements. 321-343.

Squizzato A, Romualdi E, Buller H.R and Gerdes V.E. (2007): Thyroid dysfunction and effects on the coagulation and fibrinolysis: a systematic review. Journal of Endocrinology and Metabolism. 35: 288-294.

Stallmeyer B., Kampfer H., Kolb N., Pfeilschifter J. and Frank S., (1999): The function of nitric oxide in wound repair: inhibition of inducible nitric oxide-synthase severaly impairs wound reepithelization. J. Invest. Dermatol. 113; 1090-1098.

Stuehr D.J. (2004): Enzymes of L-arginine to nitric oxide pathway. J Nutr. 134(10): 2748S-2751S.

Sun GH, Peress L and Pynnonen MA. (2013): Meta-analysis of robotic vs. conventional thyroidectomy approaches for thyroid disease. National Institute for Health Research.. www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42012003331.

Varshney R, and Kale R.K (1970): Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Biol158, 733-741.

Van Lerberghe W., Keegels G., Cornelis G., Ancona C., Mangelschots EB. and Van Balen H. (1983): Haemoglobin measurement: The reliability of Some simple techniques for use in a primary health care setting. Bulletin of the World Health Organization, 61(6): 957-965.

Wada K, Kamisaka Y, Kitano M, Kishimoto Y, Nakamoto K and Itoh T. (1996): A new gastric ulcer model induced by ischemia-reperfusion in the rat: role of leukocytes on ulceration in rat stomach. Life Sci: 59(19): PL295-301.

Wahl S.M., Wong H. and McCartney-Francis N., (1989): Role of growth factors in inflammation and repair, J.Cell Biochem. 40: 193-199.

Wiersinga WM and Touber JL (1980): The relation between gastrin, gastric acid and thyroid function disorders. Acta Endocrinol (Copenh).;95(3):341-9.

Yilmaz S., Ozan S., Benzer F., and Canatan H. (2003): Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. Cell Biochem. Funct., 21(4):325-330.

Yoshikawa T, Veda S, Naito Y, Oyemade H, Morita Y, Yoneta T and Kondo M. (1989): Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia / reperfusion in rats. Free radical research communication, 7:285-291.

*Address for correspondence:
Prof. Samuel Babafemi Olaleye
Email: ***sbolalelye@yahoo.com***
Mobile:08023255893

Conflict of Interest: None declared
Received: 27 August, 2016
Accepted: 29 October, 2016