



## Effect of thyroxine and carbimazole treatments on basal and histamine stimulated gastric acid secretion in the common African Toad (*Bufo regularis*).

Ajayi A. Folorunsho<sup>1</sup> and Olaleye S. Babafemi<sup>2</sup>

- 1- Department of Physiology, Ladoké Akintola University of Technology, Ogbomoso, Nigeria.
- 2- Department of Physiology, University of Ibadan, Ibadan, Nigeria.

Corresponding author: [ajayi22@lautech.edu.ng](mailto:ajayi22@lautech.edu.ng)

### ARTICLE INFO

#### Article History:

Received: Sept. 2, 2019  
Accepted: Sept. 29, 2019  
Online: Oct. 2019

#### Keywords:

Gastric acid secretion  
Histaminergic activity  
Hyperthyroidism  
Hypothyroidism  
Parietal cells

### ABSTRACT

Several studies have confirmed that thyroid hormones influence acid secretion particularly in mammals, but little attention is given to the influence of thyroid hormone on gastric acid secretion in amphibians. In this study, the basal and stimulated gastric acid secretions in carbimazole and thyroxine-treated common African toads were compared with controls. Thirty toads (100-120g b.w) were divided into three groups of ten toads each; Group "A" the control, Group "B" the hypothyroid group given carbimazole (5mg/250g b.w) and Group "C" the hyperthyroid group treated with thyroxine (5µg/100g b.w) treatments were carried out for 35 days by daily oral administration. Serum T<sub>3</sub>, T<sub>4</sub> and TSH levels confirmed the thyroid status of the toads. The mean Basal Gastric Acid (GAS) secretion in the control toads was significantly ( $p < 0.05$ ) higher than that of the carbimazole treated group, while the basal GAS of the thyroxine treated group was significantly ( $p < 0.05$ ) increased compared to the control. Administration of histamine intramuscularly increased significantly ( $p < 0.05$ ) the basal GAS of hyperthyroid toads, and the increment is higher than the GAS in control animals and in carbimazole treated toads. The parietal cells counts were significantly ( $p < 0.05$ ) higher in thyroxine treatment and lowered in carbimazole treated toads compared with control animals. In conclusion, increased thyroid hormone is associated with a rise in the basal secretion of gastric acid which is associated with increased histaminergic and zymogenic cell activities.

### INTRODUCTION

Thyroid hormone has been shown to influence the function of some organs including cardiovascular (Wickenden *et al.*, 1997) and nervous systems (Tejani *et al.*, 1994), liver (Daza *et al.*, 1997), growth process (Fisher *et al.*, 1982; Connors, 1994) development and reproductive systems. There is evidence that they also affect gastric acid secretion (Fateme *et al.*, 2002). Akiyama *et al.* (1982) found out that the administration of thyroxine into rats led to significant increases in serum T<sub>3</sub> level, gastric pH (reduced acid secretion) and serum gastrin level. Adeniyi and Olowookorun observed that chronic administration of thyroxine increased parietal cell mass, as well as basal acid secretion and histamine-stimulated acid secretion (Adeniyi and Olowookorun, 1989), mice also show the same trend (Olaleye and Elegbe, 2005).

The gastric acid secretion stimulator used in the present study is histamine. Histamine is released from the enterochromaffin cells of gastric mucus and has a pivotal role in the control of gastric acid secretion (Debas and Carvaial, 1994). Acetylcholine via activating histidine de-carboxylase increases the histamine release (Sandvik *et al.*, 1988; Welsh *et al.*, 1994) which in turn increases acid secretion via its receptors and cyclic adenosine monophosphate (cAMP) as a mediator in parietal cells. Sachs *et al.* (1997) observed that in rats, the released histamine from enterochromaffin cells activates the voltage-dependent  $Ca^{2+}/Cl^{-}$  channels and consequently increases gastric acid secretion.

Surveys of available literature reveal data majorly on the role of thyroid hormone on basal and histamine-stimulated acid secretion in mammals' stomach alone (Adeniyi and Olowookorun, 1989). There is insufficient information on the influence of thyroxine on basal gastric acid secretion and histamine-stimulated acid secretion in other vertebrates such as amphibian and therefore remain a question to be answered. Considering the importance of thyroxine in metamorphosis and the growth of amphibians (Laurent and Daniel, 2019), a study into the link between the thyroid gland and the digestive system (Ishizaya-Oka and Shi, 2005) and that of the possible inclusion of toad as a model of gastric secretion studies (Molero *et al.*, 1998) is very important. This study was designed to investigate the influence of altered thyroid state on basal and histamine-stimulated gastric acid secretion in the typical African toad (*Bufo regularis*) to give more insight on the mechanism by which thyroid hormones affect gastric acid secretion in amphibians.

## MATERIALS AND METHODS

Experiments were carried out on three groups (with ten adult toads per group) of toads weighing 80-120gm. Toads used in this experiment were randomly collected as described by Oyebola and Elegbe (1975). They were maintained under control ambient temperature and were allowed two weeks of acclimatization to the new environment before treatment.

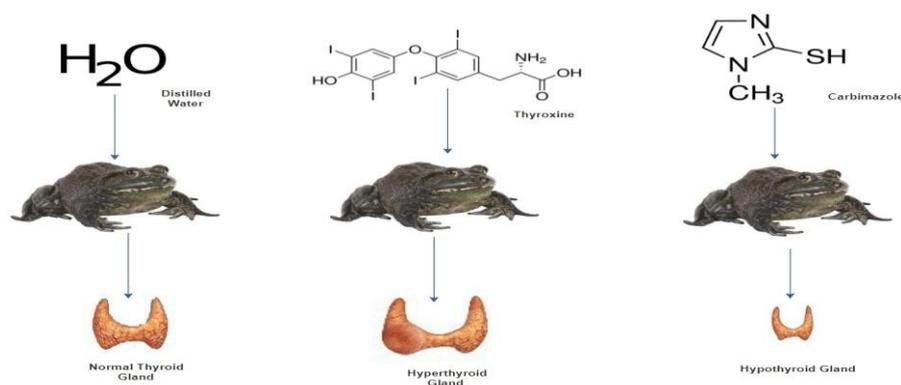


Fig. 1: Schematic of the Experimental Design.

Food and water were given *ad libitum*. The first group of toads (the control) were given distilled water, the second group (the hypothyroid) were treated with 5mg/250g b.w of carbimazole daily, while the third group (the hyperthyroid) were treated with 5 $\mu$ g/100g b.w of thyroxine daily. The drugs were administered orally in solution form for 35 days (Ajayi and Akhigbe, 2012). The weight of each toad was

recorded before the treatment, weekly as the treatment progressed and at the end of the treatment.

At the end of the experiments, blood was collected by cardiac puncture. Pooled sera samples from 5 toads from each group were analyzed for the concentrations of thyroxine ( $T_4$ ), tri-iodothyronine ( $T_3$ ) and thyroid-stimulating hormone (TSH), using Enzyme-linked Immunosorbent Assay (ELISA) Method (Surks *et al.*, 2004).

Five stomach samples from each group were used for the study on parietal cell mass, and the stomach was removed and fixed in formol saline for 24 hours, each stomach was then cut and embedded in paraffin wax, followed by sectioning with a microtome, after which the routine stain hematoxyline and eosin were applied. Parietal cells picked up dark stains. The cellular density was analyzed adopting the method of WHO (1991) in which a grid was inserted into the eyepiece of the microscope and focused through stained sections of tissues. All the cells were enumerated, and corrections were made for cells bisected by the edges or borders of the grid. The cell population was estimated as the number of cells/ $\text{mm}^2$  of tissue. The counting was done at a magnification of  $\times 400$ . Sala *et al.* (1981) mathematical correction was used to correct for the actual number of cells counted.

The *in-vivo* study of gastric acid secretion was carried out using a modification of the continuous perfusion method of Ghosh and Schild (1959) as modified by previous workers (.Adeniyi and Olowookorun, 1989; Ajayi *et al.*, 2012).

The toads were deprived of food overnight and then anaesthetized (i.p) with pentobarbital (35mg/kg). The cardiac and pyloric ends of the toad stomach were cannulated with polyethene tubing, and the stomach was perfused with Ringer's solution at room temperature, the rate of flow of the perfusate was regulated to give an effluent of  $1.0 \pm 0.1 \text{ ml/min}$ .

In each animal, four samples of basal acid secretion were first determined for all the groups, after the basal collections, the toads were injected (i.m) with 3mg/kg b.w histamine to determine the histamine-induced gastric acid secretion. Effluents from the stomachs during the experiment were collected at 15 min intervals for 90 minutes after histamine injection, and the acid content was determined by titration.

Acid secretions were expressed in  $\mu\text{eq}/15\text{min}$ . The mean acid secretions in all the groups studied were calculated, and the paired t-test was used in assessing the statistical significance of the differences. p-value of 0.05 or less was taken as statistically significant.

## RESULTS

### Weight change in altered thyroid state.

There was significant ( $p \leq 0.05$ ) reduction in percentage weight gain in thyroxine treated toads ( $-17.66 \pm 1.43\%$ ), and a significant increase in percentage weight gain in carbimazole treated toads ( $20.47 \pm 0.35\%$ ) when compared with the control ( $5.25 \pm 0.29\%$ ) as shown in Figure 2.

### Thyroid function test:

The results (Figure 3) show that chronic thyroxine treatment in toads increased  $T_3$  and  $T_4$  levels while TSH level decreased significantly ( $p \leq 0.05$ ). There were also significant ( $p \leq 0.05$ ) reduction in  $T_3$  and  $T_4$  levels and significant  $p \leq 0.05$  increase in TSH level of carbimazole treated toads when compared with the control.

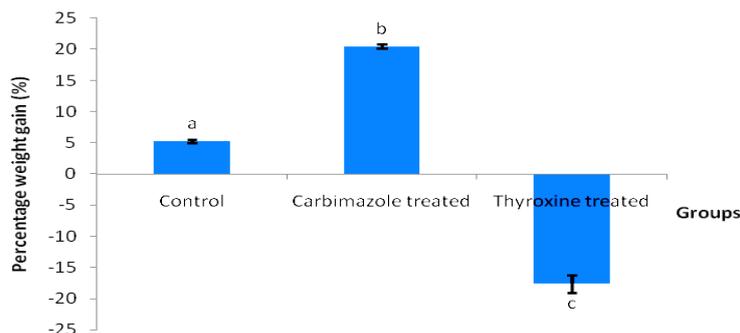


Fig. 2: Percentage weight change following treatment with carbimazole and thyroxine a,b,c = Values with different superscripts are significant at  $p \leq 0.05$

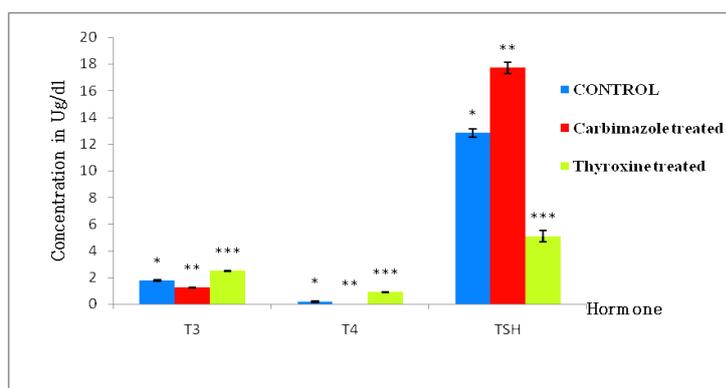


Fig. 3: Concentration of thyroid hormones following treatment with carbimazole and thyroxine  
 \* = Significant difference ( $p \leq 0.05$ ) between carbimazole treated and levothyroxine treated compared with control  
 \*\* = Significant difference ( $p \leq 0.05$ ) between carbimazole treated compared with control  
 \*\*\* = Significant difference ( $p \leq 0.05$ ) between levothyroxine treated compared with control

### Parietal Cell Count:

Table 1 shows the parietal counts of toad's stomach following treatments with carbimazole and thyroxine.

Table 1: Parietal cell Count of Control, Carbimazole and Thyroxine treated toads (cells/mm<sup>2</sup> of mucosa)

Group	Parietal cell count
Control	104 ± 0.32*
Carbimazole treated	65 ± 0.32.**
Thyroxine treated	161 ± 0.32**

\* = Significant difference ( $p \leq 0.05$ ) between carbimazole treated and levothyroxine treated compared with control

\*\* = Significant difference ( $p \leq 0.05$ ) between Carbimazole treated compared with control

\*\*\* = Significant difference ( $p \leq 0.05$ ) between levothyroxine treated compared with control

### Basal and histamine stimulated gastric acid secretion

The effect of altered thyroid state on basal and histamine stimulated gastric acid secretion in toads are shown in Table 2.

Table 2: Percentage change in basal and histamine stimulated gastric acid secretion in treated Toad

Toads	Basal secretion± SEM (µeq/15min)	Maximal response to histamine(µeq/15min)	% change over basal ± SEM
Control	1.38 ± 0.07*	2.18 ± 0.04*	61.77±0.06*
Carbimazole treated	1.13 ± 0.09***	1.92 ± 0.05***	55.86 ±0.08***
Thyroxine treated	2.24 ± 0.08 **	3.18 ± 0.09**	69.27±0.08**

\* = Significant difference ( $p \leq 0.05$ ) between carbimazole treated and levothyroxine treated compared with control

\*\*= Significant difference ( $p \leq 0.05$ ) between carbimazole treated compared with control

\*\*\*= Significant difference ( $p \leq 0.05$ ) between levothyroxine treated compared with control

Plate 1, photomicrograph section showed an excellent outline of parietal cells as indicated by black arrow, and plate 2, photomicrograph section showed large parietal cells indicated by arrow colour blue and ova zymogenic cells indicated by arrow colour brown

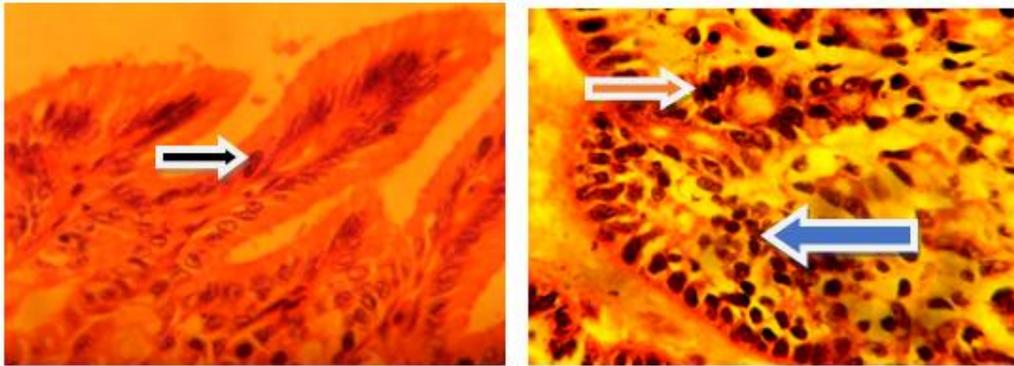


Plate 1: Control toad Parietal Cell Plate 2: Thyroxine treated toad Parietal Cell (H&E X 40)(H&E X 40).

Plate 3, showed atrophy of parietal cells indicated by a black arrow and deformed zymogenic cells as indicated by blue arrow

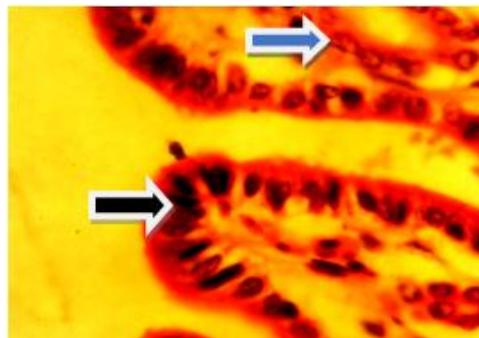


Plate 3: Photomicrograph section of stomach showing Parietal Cell Slide of carbimazole treated toad (H&E X 400)

## DISCUSSION

The results of the thyroid function tests confirmed that the toads were either hypothyroid or hyperthyroid at the time of the experiment. The serum T3 and T4 levels were in a pathophysiologic range, and this confirmed the result of the indirect tests of body weight changes, which provides indices of peripheral actions of thyroid hormones (Min *et al.*, 2018).

The study observed a significant decrease in weight in thyroxine-treated toads which contradict the work of Adeniyi and Olowookorun (1990), who reported a

significant increased liver, kidney, with the stomach and heart weight in thyroxine-treated rats. However, this is in agreement with previous studies which revealed a significantly reduced body weight gain in altered thyroid state (Ajayi and Akhigbe, 2012; Ajayi *et al.*, 2013). The result showed the role of the average concentration of thyroid hormone in optimal growth (Ajayi and Akhigbe, 2012).

The relationship between thyroid hormones and its altered states on gastric acid secretion has been studied in several animal species and human (Akiyama *et al.*, 1982; Kazuhiko *et al.*, 2006). The reduced secretion in carbimazole treatment and increased secretion in tyrosine treatment is in agreement with the studies on rats (Akiyama *et al.*, 1982; Adeniyi and Olowookorun, 1990); in human subjects (Kazuhiko *et al.*, 2006; Fatemeh and Saleh, 2012). Adeniyi and Olowookorun (1990) suggested that thyroid hormones regulate basal and secretagogue stimulated acid secretion via their effects on parietal cell mass (Adeniyi and Olowookorun, 1989). Kayode and Michael (1998) also explained that an increase in the mass of parietal cells increases mitotic activity and causes an increase in acid secretion in thyroxine treatment, while thyroidectomy inhibits parietal cell mitotic activity and decreases acid output.

The significant increase in gastric acid output after the administration of histamine in this study have been observed in earlier studies on amphibian species (Oyebola and Alada, 1992), but no information on the effect in altered thyroid state. The present study showed that thyroxine further increased the effect of histamine on gastric acid secretion. The result also shows the same pattern with the increased parietal cell count observed in this study. Parietal cells contain histamine H<sub>2</sub>-type of receptors for the endocrine control of acid secretion (Forte and Zhu, 2010). The presence of histamine- H<sub>2</sub> receptors in the gastric mucosa of bullfrog has been described (Watanabe and Goto, 1975). It was observed that the regulation of gastric acid secretion by histamine is through the activation of adenylyl cyclase, which therefore increases the production of intracellular CAMP (Zeng and Sachs, 1998). Thus, it was suggested that thyroid hormone is capable of increasing gastric acid secretion locally by stimulating the release of histamine from the enterochromaffin-like (ECL) cells (Kitamura *et al.*, 1999).

The histology of the stomach of an animal may be responsible for the type of diets taken by the animal, and the architectural arrangement of its structures gives an interpretation their mechanism of adaptation to these diets (Hildebrand and Goslow, 2001; Ofusori *et al.*, 2008).

The large parietal cells and ova zymogenic cells as a result of thyroxine treatment in toad is different when compared with the work of Akiyama *et al.* (1989) which reported that parietal cells of rats remain normal after treatment with thyroxine. Also, carbimazole treatment showed atrophy of parietal cells and deformed zymogenic cells in the toad.

The parietal cells of rats secrete gastric acid; while the zymogenic cells of rats produce pepsin (Kayode and Michael, 1989). Previous studies believe that in amphibian the zymogenic cells produce both acid and pepsin (Forte and Zhu, 2010). The results of this study suggest that both parietal cells and zymogenic cells may be responsible for acid secretion in the toad, which could be responsible for the prominent secretion of acid recorded.

## CONCLUSION

In conclusion, this study showed that thyroxine is associated with a significant rise in basal gastric acid secretion from both parietal cell count rise with an increase in the size and zymogenic cell activities. While carbimazole treatment reduced the secretion due to the reduced size of parietal cells count and activities of zymogenic cells. This study put the toad in a position as a possible model for gastric acid secretion study.

## REFERENCES

- Adeniyi, K.O. and Olowookorun, M.O. (1989). Gastric acid secretion and parietal cell mass: effects of thyroidectomy and thyroxine. *AJP. Gastrointestinal and liver physiology*, 256(6): 975-978.
- Adeniyi, K.O. and Olowookorun, M.O. (1990). Effect of thyroidectomy and thyroxine administration on the growth of vital organs in rats. *Nig. J. Physiol. Sci.*, 4:64-66.
- Ajayi, A.F. and Akhigbe, R.E. (2012). Implication of altered thyroid state on liver function. *Thyroid Research and Practice*, (9)3:84-87.
- Ajayi, A.F.; Akhigbe, R.E. and Ajayi, L.O. (2013). Hypothalamic-pituitary-ovarian axis in thyroid dysfunction. *West Indian Medical Journal*, 62(9):835-838.
- Ajayi, A.F.; Akhigbe, R.E.; Iyiola, T.O.; Adewumi, O.M. and Olaleye, SB. (2012). Gastric secretagogue action of *Cryptolepis sanguinolenta* in the perfused stomach of anesthetized rats. *Int J Med Biomed Res.*, 1(1): 62-67.
- Akiyama, T.; Kishimoto, S. and Miyaji, K. (1982). Gastric acid secretion serum gastrin and parietal cell histology in rat hyperthyroidism. *Gastroentrol. Journal*, 17(1): 50-55.
- Connors, J.M. (1994). Thyroid and antithyroid drugs. In: Craig CR, Stitzel RE, editors. *Modern pharmacology*. 4th edition. Boston, Massachusetts: Little, Brown Co., 775-85.
- Daza, F.J.; Parrilla, R. and Martin Requero, A. (1997). Influence of thyroid status on hepatic alpha-1-adrenoreceptor responsiveness. *Am J Physiol.*, 273:1065-72.
- Debas, H.T. and Carvajal, S.H. (1994). Vagal regulation of acid secretion and gastrin release. *Yale J Biol Med.*, 67: 145-151.
- Fatemeh, N.R. and Saleh, Z. (2002). Gastric acid and pepsin secretions in rats. *Ann Saudi Med.*, 22(5-6): 308-311.
- Fatemeh, N.R.; Saleh, Z.; Mohammad, K.G. and Jalal, V. (2002). Effect of thyroid hormones on distension-induced gastric acid and pepsin secretions in rats. *Ann Saudi Med.*, 22(5-6): 308-311.
- Fisher, D.A.; Hoath, S. and Lakshmanan, J. (1982). The thyroid hormone effects on growth and development may be mediated by growth factors. *Endocrinol Exp.*, 16:259-71.
- Forte, J.G. and Zhu, L. (2010). Apical Recycling of the Gastric Parietal Cell H, K-ATPase. *Annu Rev Physiol.*, 72:273-96.
- Ghosh, M.N. and Schild, H.O. (1959). Continuous recording of gastric acid secretion in the rat. *Brit. J. Pharmacology*, 13, 54-61.
- Hildebrand M. and Goslow, G. (2001). *Analysis of Vertebrate Structure*. 5<sup>th</sup> ed. New York: John Wiley & Sons Inc., 201-17.

- Ishizuya-Oka, A. and Shi, Y.B. (2005). Molecular mechanisms for thyroid hormone-induced remodeling in the amphibian digestive tract: a model for studying organ regeneration *Dev. Growth Differ.*, 47 (9):601-607.
- Kayode, O.A. and Michael, O.O. (1989). Gastric acid secretion and parietal cell mass: effects of thyroidectomy and thyroxine. *Am J Physiol.*, 256:G975-978.
- Kazuhiko, A.; Koichiro, U. and Kunihiko, I. (2006). Gastric secretion in hyperthyroidism. *Surgery Today*, 12(3):198-202.
- Kitamura, M.; Sugamoto, S.; Kawauchi, S. and Kato, S. (1999). Takeuchi K. Modulation by nitric oxide of acid secretion induced by gastric distension in rats: Enhancement by NO synthase inhibitor. *Pharmacol and Expt. Ther.*. 298:181-187.
- Laurent, M.S. and Daniel R.B. (2019). Insufficiency of Thyroid Hormone in Frog Metamorphosis and the Role of Glucocorticoids, *Front Endocrinol (Lausanne)*. 10: 287.
- Min, J.K.; Sun, W.C.; Sumin, C.; Dal, L.Ju.; Do, J.P. and Young, J. P. (2018). Changes in Body Compositions and Basal Metabolic Rates during Treatment of Graves' disease. *International Journal of Endocrinology*, ID 9863050, 8 pages.
- Molero, M.; Hernandez, I.M.; Lobo, P.; Cardenas, P.; Romero, R. and Chacin, J. (1998). Modulation by nitric oxide of gastric acid secretion in toads. *Acta Physiologica Scandinavica*, 164: 229-36.
- Ofusori, D.A.; Caxton-Martins, E.A.; Keji, S.T.; Olayinka, P.O.; Abayomi, T.T. and Ajayi, S.A. (2008). Microarchitectural adaptations in the stomach of African tree Pangolin (*Manis tricuspis*). *Int J Morphol.*, 26:701-5.
- Olaleye, S.B. and Elegbe, R. A. (2005). Catecholamine potentiates the effect of thyroid hormones on intestinal absorption of glucose in the rats. *Afri. J. Med Sci.*, 34: 177-183.
- Oyebola, D.D.O and Elegbe, R.A. (1975). Gastrin activity in the stomach extracts of *bufo regularis* (the common African Toad). *Comp. Biochem. & Physiol.*, 52A: 209-211.
- Oyebola, D.D.O. and Alada, A.R.A. (1992). High doses of Histamine required for gastric acid secretion in the common African toad *Buffo regularis*, *Isr. J. Med. Sci.*, 28: 731-733.
- Sachs, G.; Zeng, N. and Prinz, C. (1997). Physiology of isolated gastric endocrine cells. *Annu Rev Physiol.*, 59: 243-256.
- Sala, M.A.M.; Matheus, M. and Valer, V.A. (1981). A new stereological method for estimation: the thickness of a cellular layer on random sections. *Mikroskopile Wien*, 38(516):127-30.
- Sandvik, A.K.; Kleveland, P.M. and Waldum, H.L. (1988). Muscarinic M2 stimulation releases histamine in the totally isolated, vascularly perfused rat stomach. *Scand J Gastroenterology*, 23:1049-1056.
- Surks, M.I.; Ortiz, E.; Daniels, G.H.; Sawin, C.T. and Col, N.F. (2004). Subclinical thyroid disease: Scientific review and guidelines for diagnosis and management. *JAMA*, 291(2):228-38.
- Tejani Butt, S.M. and Yang, J.A. (1994). Time course of altered thyroid status on the noradrenergic system in rat brain by quantitative autoradiography. *Neuroendocrinology*, 59:235-44.
- Watanabe, K. and Goto, Y. (1975). Dibenamide blockade on gastric acid secretion in vitro and its protection by histamine antagonist. *Br. J. Pharmacol.*, 30:133-139.

- Welsh, N.J.; Shankley, N.P. and Black, J.W. (1994). Comparative analysis of the vagal stimulation of gastric acid secretion in rodent isolated stomach preparation. *Br J Pharmacology*, 112: 93-96.
- Wickenden, A.D.; Kaprielian, R.; Parker, T.G.; Jones O.T. and Backx, P.H. (1997). Effects of development and thyroid hormone on K<sup>+</sup> currents and K<sup>+</sup> channel gene expression in rat ventricle. *J Physiol.*, 504:271-86.
- World Health Organization (WHO) (1991). Calibrating the microscope. In: *Basic laboratory methods in medical parasitology*, Geneva.
- Zeng, N. and Sachs, G. (1998). Properties of isolated gastric entero-chromaffin-like cells. *Yale Journal of Biology and Medicine*. 71:233-246.