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Vascular Modulatory Roles Of The Sex Hormones And Thyroxine In Tadalafil-Treated Wistar Rats.

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ABSTRACT

Current research effort on gastric ulcer focuses on agents that improve gastro-protection. We have previously established that Tadalafil, a phosphodiesterase V inhibitor currently used to treat pulmonary hypertension and erectile dysfunction, ameliorated the effects of indomethacin-induced ulcerations in rats. In this study, we investigated the influence of the sex hormones and thyroxine on ulcerogenesis, gastric acid secretion and mucus production in Tadalafil-treated ulcerated rats. Adult male and female Wistar rats (140-160 g) were used. Gastric ulcer was induced in fasted rats using indomethacin (40 mg/Kg, p.o.). Total gastric acid was measured by pyloric ligation and the continuous perfusion method, respectively. Gastric mucus content was estimated using spectrophotometry. Ulcer scores were assessed using planimetry and stomach tissue sections evaluated by microscopy. Data were analysed using ANOVA followed by Newman Keul's post-hoc test at $\alpha_{0.05}$. Mean ulcer score and ulcer area were significantly reduced by tadalafil and the combination of tadalafil and either of oestrogen or thyroxine. Total titratable and stimulated acid secretions were significantly decreased by tadalafil and oestrogen. This anti-secretory and mucogenic gastro-protective effect is enhanced by thyroxine and oestrogen, but not by testosterone.

Keywords: Tadalafil, Gastric ulcer, Thyroxine, Sex hormones, Phosphodiesterase inhibitor, gastro-protection.

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INTRODUCTION

Gastrointestinal tract disorders are a major health concern in any given populace. Ulcers, and more specifically, peptic ulcer, remain one of the most prevalent. They are usually characterized by laceration of the mucosa of the stomach or duodenum (gastric or duodenal ulcer). Peptic ulcers result from a disturbance of the balance between aggressively damaging forces and factors responsible for maintaining the defence of the gastric mucosa. Repeated exposure to these aggressive factors contributes significantly to the increased risk for ulcer development. Such factors include chronic stress, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), nicotine use, gastric acid secretion, bacteria such as the Helicobacter pylori among others.

Phosphodiesterase V inhibitors are drugs currently used to treat pulmonary hypertension and erectile dysfunction. This group of drugs work by increasing the regional blood flow in response to increased cyclic guanosine monophosphate (cGMP) synthesis (PDE V enzymes break down cGMP). cGMP mediates many of the biological actions attributed to nitric oxide (NO) which is a proven vasodilator that increases blood flow in tissues. In establishing the underlying mechanisms of indomethacin-induced gastric mucosal damage, it was showed that a decrease in mucosal blood flow can lead to mucosal erosions [1]. An earlier work showed that Sildenafil, a type V phosphodiesterase inhibitor, increases cGMP concentrations in the GIT which in turn promotes the endogenous synthesis of NO [2].

The role of sex hormones in ulcerogenesis is still a much debated issue. While several authors have reported anti-ulcerogenic effects of the sex hormones (especially the female ones) a few other researchers have highlighted their ulcerogenic properties in animal models. This study was carried out to investigate the influence of the sex hormones and thyroxine on ulcerogenesis, gastric acid secretion and mucus production in Tadalafil-treated ulcerated rats

MATERIALS AND METHODS

ANIMALS

Male and female Wistar rats weighing between 140-160g were used. They were procured from the Central Animal House, College of Medicine, University of Ibadan, Ibadan. They were housed in clean, well-ventilated polypropylene cages with comfortable ambient temperature. They were acclimatized for at least a period of two weeks before any experimental work was done and maintained under standard condition of 12 hours of alternating light and dark cycle. The animals were fed with standard rat chow and were allowed water *ad libitum*. The animals were treated humanely under globally accepted guidelines for good laboratory practice and the principles of laboratory animal care.

EXPERIMENTAL DESIGN

The following investigations were carried out;

- Determination of ulcer indices (mean ulcer score and index) and dimensions in the various treatment groups. We tested the role of thyroidectomy, ovariectomy, and castration with subsequent respective hormonal replacements on experimentally-induced ulceration.
- Estimation of total gastric acid output by volumetric analysis
- Estimation of basal and stimulated gastric acid secretion using a modified method of continuous perfusion technique [3].

Estimation of gastric mucus production by spectrophotometry

Animals were divided according to the following experimental groups:

- Group I (Control) – distilled water 0.2ml/100g BW
- Group II- Thyroidectomised
- Group III - Tadalafil 10mg/kg BW
- Group IV- Thyroidectomised + Tadalafil 10mg/kg BW
- Group V– Hormonal replacement (Thyroxine) + Tadalafil 10mg/kg BW

Group I-V was repeated for the castrated and ovariectomised studies.

EXPERIMENTAL PROCEDURES

Ulcer Induction

Ulcer was induced using indomethacin, a known non-steroidal anti-inflammatory drug. Animals were fasted for 24 hours before the start of experimentation but had free access to clean tap water ad libitum. The Control group received Indomethacin (40 mg/kg bw, p.o). All other groups received Indomethacin (40 mg/kg bw, p.o) 30 min after receiving their respective pre-treatments. All animals were euthanized 4 hours later with ether overdose; the stomachs were removed and assessed for ulcer lesions by planimetry [4].

Gastric Anti-Ulcer Assays

Determination of Ulcer scores

Ulcers were independently assessed and scored by two observers using the following criteria-

- Normal stomach – 0
- Punctuate/pin-point ulcer - 0.5
- Two or more small haemorrhagic ulcers – 1.0
- Ulcers larger than 2mm – 2.0 [5]

Index of Ulceration

Paul's Index was used as ulcer index [6]. This index is expressed as the formula:

$$(M \times N) /100$$

where
M= mean number of ulcers per rat in the group,
N= percentage of rats with ulcer in the group.

Percentage Inhibition

$$\textbf{\% Inhibition} = [I_{control} – I_{treated} /I_{control}] X 100$$

Determination of Ulcer Dimensions and Histo-morphometric Assessment

Histological profiling of the ulcerated gastric mucosa was carried out using Periodic Acid Schiff staining (PAS) [7]. Ulcer dimension (Area, Depth and Width) determinations were done using micrometric software (Motic 2000). Stomachs fixed with 10% formalin and embedded in paraffin were cut into 10-16µm sections in cryostat and then mounted in an automated microtome. The gastric tissue integrity (mucosa-submucosa) was assessed for damage.

Estimation of gastric acid output

Total titratable gastric acid

Four hours after ulcer induction, each stomach was ligated at the cardiac and pyloric end and filled with 12mL of distilled water. The stomach was shaken to mix the gastric contents effectively. The infused fluid (10 mL) was withdrawn with a syringe and then titrated against 0.01N NaOH for total gastric acid estimation.

Basal and Histamine-stimulated gastric acid secretion

This was based on a modification of the method by Ghosh and Schild [3]. The animals were fasted overnight but had free access to water. The animals were given their respective pre-treatments and then anaesthetized with ketamine, 7.5mg/100g BW. Basal effluent was collected for 20 min after which histamine (1mg/kg i.m) was administered. Gastric effluent was collected every 10 min over the next one hour and the acidity assayed by titration as described above.

MUCUS PRODUCTION STUDY

Briefly, the excised stomachs were soaked for two hours in 0.1% alcian blue. Excessive dye was removed by two successive rinses in 0.25M sucrose at 15 minutes and 45 minutes. Excess dye complexed with mucus was extracted by mixing with 0.5M $MgCl_2$ and centrifuged for 5 minutes. The optical density of the aqueous supernatant layer was read with a spectrophotometer at a wavelength of 580nm against a buffer blank. The quantity of alcian blue extract per gram wet stomach tissue was calculated from a standard curve and the result expressed as microgram (µg) alcian blue per gram wet tissue [8].

STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM. Statistical difference between test groups and control group was calculated using one way ANOVA. Newman Keul's post-Hoc test was done. $p<0.05$ was considered as significant.

RESULTS

EFFECT OF THYROIDECTOMY AND THYROXINE REPLACEMENT ON MEAN ULCER SCORE AND ULCER INDEX AND PERCENTAGE INHIBITION

The effect of thyroidectomy (TMZ) and thyroxine replacement on mean ulcer score and ulcer index of indomethacin-treated rats is shown in Table 1. Thyroidectomy increased the ulcer score (16.83 ± 3.12). When compared to Control (14.42 ± 2.93) tadalafil (TAD) inhibited or reversed this effect as ulcer score was significantly lower when thyroidectomised rats were administered tadalafil (11.83 ± 1.63, $p<0.05$) and in the TAD only group (10.64 ± 2.12). Co-administration of tadalafil with thyroxine to thyroidectomised rats (Group 5) produced significant reduction in ulcer scoring when compared with both the Control and TAD only group (5.83±1.57, $p<0.05$).

TABLE 1: EFFECT OF THYROIDECTOMY (TMZ) AND THYROXINE REPLACEMENT ON MEAN ULCER SCORE AND ULCER INDEX IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	MEAN ULCER SCORE	PAUL'S INDEX
Control (euthyroid)	14.42±2.93	14.42
TMZ	16.83±3.12	16.83
TAD 10 mg/kg	10.64±2.12*	9.81
TMZ + TAD 10 mg/kg	11.83±1.63*	10.65
TMZ + T4 + TAD 10 mg/kg	5.83±1.57*#	5.25

Data presented as Mean±SEM (n=5)
* - significant at $p<0.05$ when compared with Control
# - significant at $p<0.05$ when compared with TAD only

The pattern of ulcer inhibition (percentage inhibition) is shown in Figure 1. Thyroidectomy made the degree of ulceration worse as depicted by the negative deflection below the baseline (-16.71%). Tadalafil 10mg/kg had a 31.96% inhibition of ulceration, an effect that was slightly inhibited by thyroidectomy (26.14%). Co-administration of tadalafil with thyroxine to thyroidectomised rats yielded a greater inhibition (63.59%) when compared to Control in a pattern similar to the ulcer scoring result.

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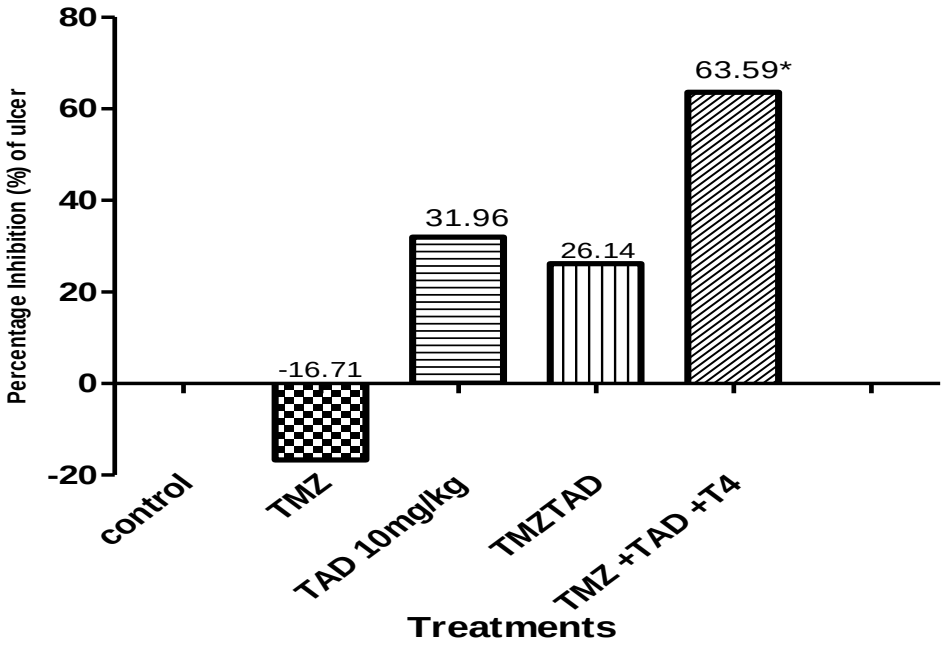

Fig. 1: Effect of thyroidectomy (TMZ) and thyroxine replacement on percentage inhibition of ulcer in indomethacin-induced ulceration.

* - significant at p<0.05 when compared with Control

EFFECT OF CASTRATION AND TESTOSTERONE REPLACEMENT ON MEAN ULCER SCORE, ULCER INDEX AND PERCENTAGE INHIBITION

The pattern of ulcer scoring in normal and castrated rats treated with indomethacin is shown in Table 2. Ulcer scoring was higher in castrated rats (15.00±2.12) when compared to Control (14.42± 2.93). Tadalafil significantly lowered ulceration when compared with Control (9.33±1.92). This ulceration inhibition was reversed in castrated rats treated with TAD 10mg/kg (11.83±1.41). Testosterone (TST) replacement in combination with tadalafil in castrated rats produced no significant inhibition of ulcer score (13.17±1.14).

TABLE 2: EFFECT OF CASTRATION AND TESTOSTERONE (TST) REPLACEMENT ON MEAN ULCER SCORE AND ULCER INDEX IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	MEAN ULCER SCORE	PAUL'S INDEX
Control	14.42±2.93	14.42
Castrated	15.00±2.12	15.00
TAD 10 mg/kg	9.33±1.92*	11.81
Castrated + TAD 10 mg/kg	11.83±1.41	10.65
Castrated + Testosterone + TAD 10 mg/kg	13.17±1.14	11.85

Data presented as Mean±SEM (n=5)

* - significant at p<0.05 when compared with Control

The percentage inhibition of ulceration in castrated, uncastrated and testosterone-replaced rats is shown in FIGURE 2. Castration made ulceration worse as shown by the negative deflection (below baseline) with a percentage inhibition of -4.02 thus showing exacerbation of the ulceration process. Tadalafil produced an inhibition of ulceration in intact animals (18.10%) compared to Control while percentage inhibition was lowered in castrated rats treated with TAD (9.14%) and those administered with a combination of tadalafil and testosterone (8.66%).

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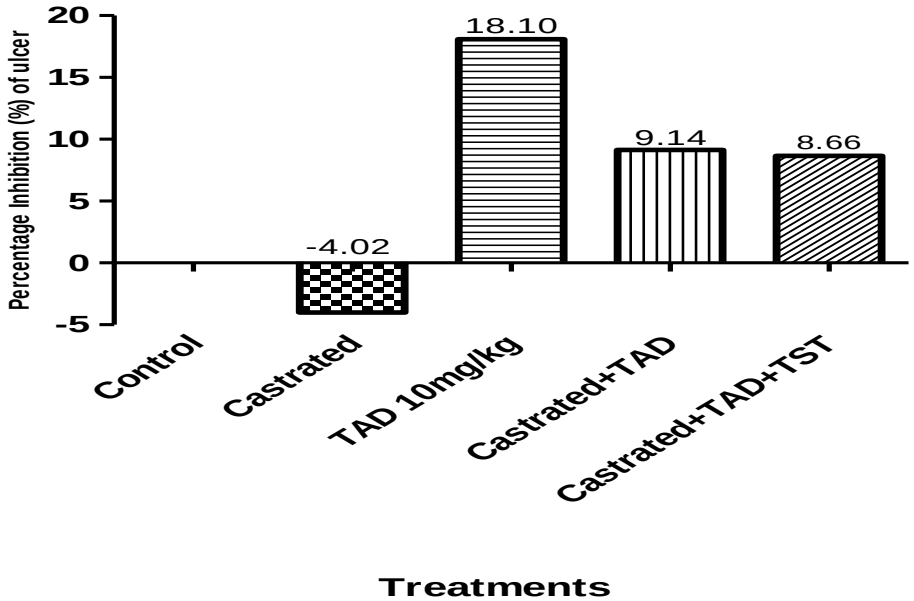

Figure 2: Effect of castration and testosterone replacement on percentage inhibition of ulcer in indomethacin-induced ulceration.

EFFECT OF OVARIECTOMY AND OESTROGEN REPLACEMENT ON MEAN ULCER SCORE, ULCER INDEX AND PERCENTAGE INHIBITION

The pattern of ulceration induced with indomethacin in normal and ovariectomised rats is shown in Table 3. Mean ulcer scores of both the Control group (15.42±1.88) and ovariectomised group (15.08±0.89) compared favourably with each other with no significant difference when tested. TAD-only treated animals had a significantly lower mean ulcer score compared to control (9.93±2.51, $p<0.05$). Ovariectomy increased mean ulcer score in TAD-treated rats. Oestrogen replacement in ovariectomised rats treated with tadalafil significantly lowered the mean ulcer score (7.92±1.14, $p<0.05$).

TABLE 3: EFFECT OF OVARIECTOMY AND OESTROGEN REPLACEMENT ON MEAN ULCER SCORE AND ULCER INDEX IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	MEAN ULCER SCORE	PAUL'S INDEX
Control	15.42±1.88	15.42
Ovariectomised	15.08±0.89	15.08
TAD 10 mg/kg	9.93±2.51*	9.81
Ovariectomised + TAD 10 mg/kg	13.92±1.77	12.53
Ovariectomised+ oestrogen + TAD 10mg/kg	7.92±1.14*	7.13

Data presented as Mean±SEM (n=5)
* - significant at $p<0.05$ when compared with Control

The percentage inhibition of ulceration in normal, ovariectomised and oestrogen-replaced animals is shown in Figure 3. Ovariectomy produced no significant inhibition of ulceration when compared with control unlike the TAD-only treated group which had a percentage ulcer inhibition of 36.40%. Ovariectomised TAD-treated animals had an ulcer inhibition percentage of 18.74% when compared to control but this is lower compared to the inhibition in intact animals administered with TAD-only. Oestrogen replacement in combination with tadalafil treatment produced a significant ulcer inhibition percentage of 48.64%.

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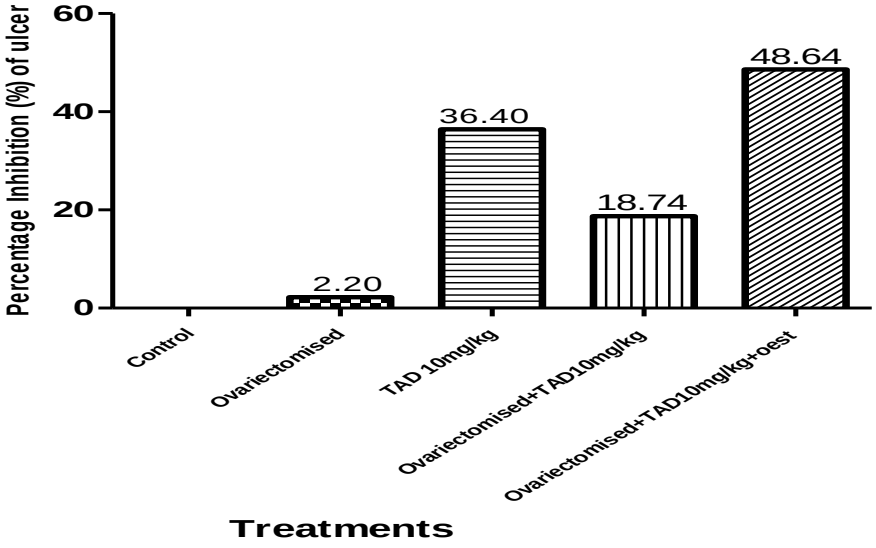

Figure 3. Effect of ovariectomy and oestrogen replacement on percentage inhibition of ulcer in indomethacin-induced ulceration.

DETERMINATION OF ULCER DIMENSIONS (AREA AND DEPTH)

Effect of Thyroidectomy and Thyroxine Replacement on Ulcer Area and Depth

The ulcer area (μm^2) and depth (μm) as shown in Table 4, were both decreased in all treated groups when compared to control (353.50±4.80 μm^2 and 4465.38±95.10 μm respectively, $P<0.05$). Tadalafil significantly reduced both area and depth (124.11±6.81 μm^2 and 780.58±24.62 μm respectively, $P<0.01$). This effect was reduced in the thyroidectomised group treated with tadalafil (192.67±1.65 μm^2 and 519.74±7.22 μm). Thyroxine in combination with tadalafil in thyroidectomised rats also had a significant reduction of ulcer dimensions when compared to control ($P<0.01$).

TABLE 4: EFFECT OF THYROIDECTOMY AND THYROXINE REPLACEMENT ON ULCER AREA AND DEPTH IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	Area (μm^2)	Depth (μm)
Control	353.50±4.80	4465.38±95.10
Thyroidectomised	245.01±3.98	3848.05±312
TAD 10 mg/kg	124.11±6.81*	780.58±24.62*#
Thyroidectomised + TAD 10 mg/kg	192.67±1.65*	519.74±7.22*#
Thyroidectomised +T4 + TAD 10 mg/kg	132.67±1.21*#	428.87±4.66*#

Data presented as Mean±SEM (n=5)
* - significant at $p<0.05$ when compared with Control
# - significant at $p<0.05$ when compared with TAD only

EFFECT OF CASTRATION AND TESTOSTERONE REPLACEMENT ON ULCER AREA AND DEPTH

Tadalafil significantly reduced the ulcer area and depth in the uncastrated rats (124.11±6.81μm^2 and 780.58±24.62μm respectively, $P<0.05$). The findings of this study showed no significant difference on the effect of testosterone withdrawal and/or supplementation on ulcer dimensions (Table 5). Thus neither castration nor testosterone supplementation with tadalafil aggravated nor diminished significantly the effects of indomethacin on the gastric mucosa. The observed significant reduction in the ulcer depth of castrated rats

treated with tadalafil may be attributed solely to the established inhibitory effects of tadalafil on ulcerogenesis.

TABLE 5: EFFECT OF CASTRATION AND TESTOSTERONE REPLACEMENT ON ULCER AREA AND DEPTH IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	Area (μm^2)	Depth (µm)
Control	353.50±4.80	4465.38±95.10
Castrated	445.01±3.98	3848.05±30.48
TAD 10 mg/kg	124.11±6.81 *#	780.58±24.62 *#
Castrated+ TAD 10 mg/kg	252.67±2.88	1519.74±7.22 *#
Castrated+Testosterone + TAD 10 mg/kg	286.76±4.21	2428.87±5.57

Data presented as Mean±SEM (n=5)
* - significant at p<0.05 when compared with Control
# - significant at p<0.05 when compared with TAD only

EFFECT OF OVARIECTOMY AND OESTROGEN REPLACEMENT ON ULCER AREA AND DEPTH

The ulcer area (μm^2) and depth (µm) in normal and ovariectomised rats is shown in Table 6. Ovariectomy increased both the ulcer area (381.01±4.00μm^2) and depth (5105.05±46.44µm) when compared to control (320.30±4.80μm^2 and 4144.18±22.94µm respectively). Tadalafil significantly reduced both area and depth (124.11±6.81 μm^2 and 780.58±24.62 µm respectively, P<0.05). This effect was reduced in the ovariectomised group treated with tadalafil (210.33±5.42μm^2 and 1718.13±47.63µm respectively). Oestrogen in combination with tadalafil in ovariectomised rats also had a significant reduction of ulcer dimensions when compared to control (156.36±1.79µm2 and 1483.53±6.98µm respectively, P<0.05).

TABLE 6: EFFECT OF OVARIECTOMY AND OESTROGEN REPLACEMENT ON ULCER AREA AND DEPTH IN INDOMETHACIN-INDUCED ULCERATION

TREATMENT	Area (μm^2)	Depth (µm)
Control	320.30±4.80	4144.18±22.94
Ovariectomised	381.01±4.00	5105.05±46.44
TAD 10 mg/kg	124.11±6.81 *	780.58±24.62 *#
Ovariectomised+ TAD 10 mg/kg	210.33±5.42 *	1718.13±47.63 *#
Ovariectomised+Oestrogen+ TAD 10 mg/kg	156.36±1.79 *	1483.53±6.98 *#

Data presented as Mean±SEM (n=5)
* - significant at p<0.05 when compared with Control
# - significant at p<0.05 when compared with TAD only

ESTIMATION OF GASTRIC ACID OUTPUT

Result from this study (Table 7) showed that gastric acid secretion in response to thyroidectomy (9.3±0.44 mEq/L), thyroxine replacement (8.8±0.38 mEq/L) and tadalafil administration (8.5±1.35 mEq/L) did not vary significantly when compared to control (10.5±1.52 mEq/L, p<0.05). In the Ovariectomy and oestrogen replacement study however (Table 8), tadalafil produced a significant reduction in gastric acid secretion (8.5±1.35 mEq/L) when compared to control (12.5±2.12 mEq/L, p<0.05). The combination of oestrogen and tadalafil produced a significant reduction in gastric acid secretion (5.7±0.69 mEq/L) when compared to control and the tadalafil-only treated group.

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TABLE 7: EFFECT OF THYROIDECTOMY AND THYROXINE REPLACEMENT ON TOTAL TITRATABLE GASTRIC ACID SECRETION IN INDOMETHACIN-INDUCED ULCERATION

Treatment	Total Acid (mEq/L)
Control	10.5±1.52
Thyroidectomised	9.3±0.44
TAD 10 mg/kg	8.5±1.35
Thyroidectomised + TAD 10 mg/kg	7.5±0.66
Thyroidectomised +T4 + TAD 10 mg/kg	8.8±0.38

Data presented as Mean±SEM (n=5)

TABLE 8: EFFECT OF OVARIECTOMY AND OESTROGEN REPLACEMENT ON TOTAL TITRATABLE GASTRIC ACID SECRETION IN INDOMETHACIN-INDUCED ULCERATION

Treatment	Total Acid (mEq/L)
Control	12.5±2.12
Ovariectomised	14.6±1.35
TAD 10 mg/kg	8.5±1.35*
Ovariectomised + TAD 10 mg/kg	9.8±0.11
Ovariectomised + Oestradiol + TAD 10mg/kg	5.7±0.69#*

Data presented as Mean±SEM (n=5)

* - significant at $p<0.05$ when compared with Control

- significant at $p<0.05$ when compared with TAD only

EFFECT OF THYROIDECTOMY, OVARIECTOMY, THYROXINE AND OESTROGEN REPLACEMENT ON BASAL AND HISTAMINE-STIMULATED GASTRIC ACID SECRETION IN INDOMETHACIN-INDUCED ULCERATION

The pattern of basal and histamine stimulated gastric acid secretion in response to either thyroidectomy and thyroxine replacement or ovariectomy and oestrogen replacement in response to indomethacin treatment is shown in Figure 4 and Figure 5.

Histamine potentiated gastric acid secretion in both studies as there was a significant increase in acid secretion following histamine administration in the control. Tadalafil suppressed the histamine gastric acid secreting potentiating activity. Thyroidectomy also failed to potentiate histamine activity. However, there is a significant potentiation of histamine action in response to thyroxine administration, which was not observed in other treated groups (FIGURE. 4). This potentiating action was not suppressed by co-administration with Tadalafil or thyroidectomy.

FIGURE 5 shows the pattern of basal and histamine stimulated gastric acid secretion in ovariectomised and oestrogen replaced animals. Ovariectomy potentiated histamine action resulting in elevated acid secretion. There is a significant reduction in the level of histamine activity when tadalafil was co-administered with oestrogen compared to control.

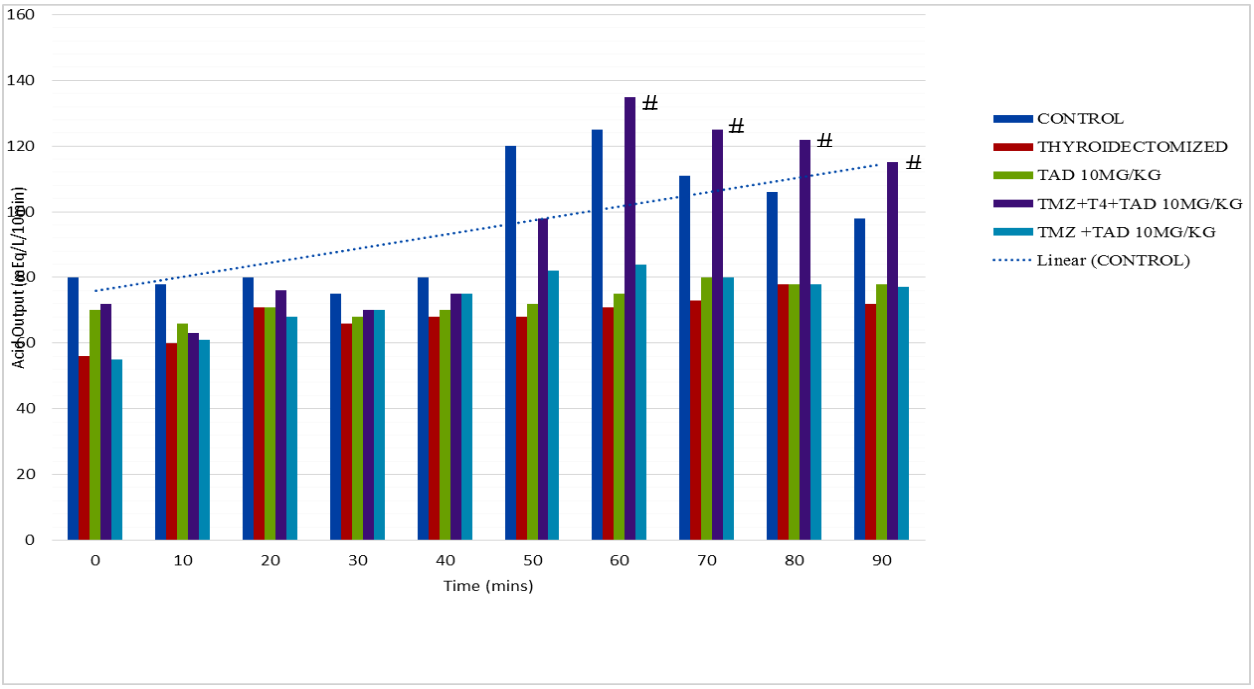

Fig. 4: Effect of ovariectomy and oestrogen replacement on basal and histamine-stimulated gastric acid secretion in indomethacin-induced ulceration

Data presented as Mean±SEM (n=5)

- significant at $p<0.05$ when compared with Control

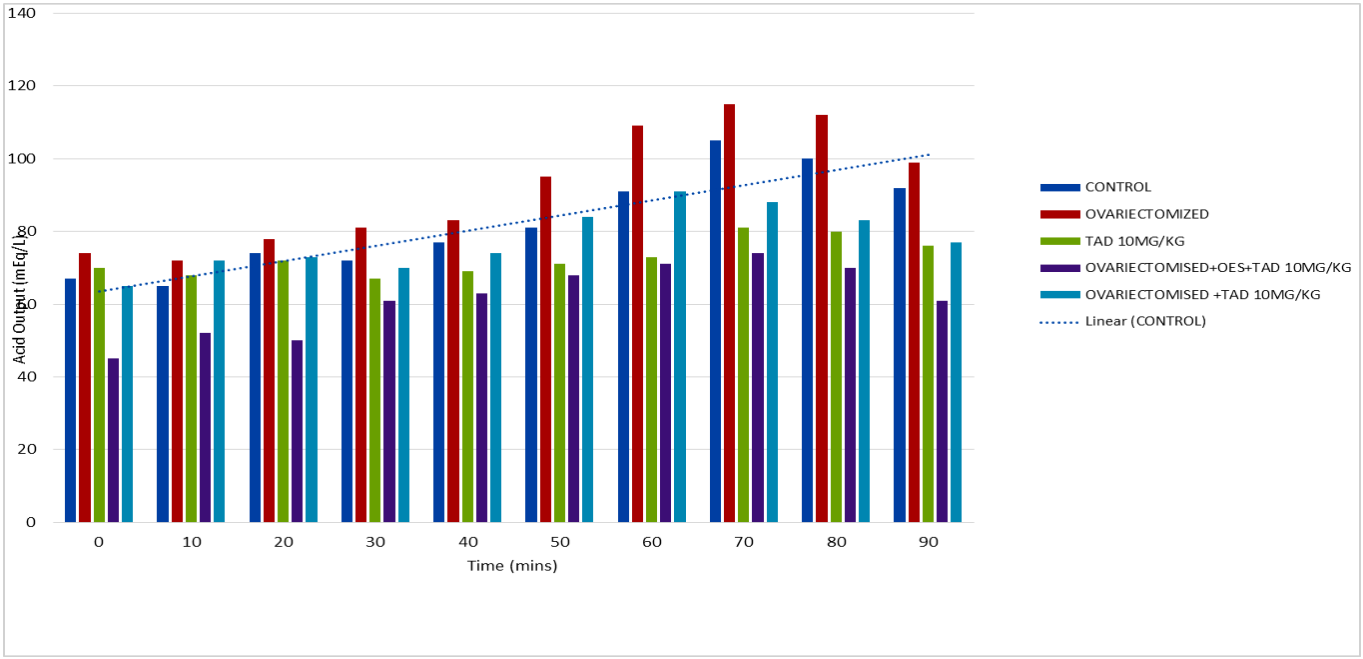

Fig. 5: Effect of Ovariectomy and oestrogen replacement on basal and histamine-stimulated gastric acid secretion in indomethacin-induced ulceration

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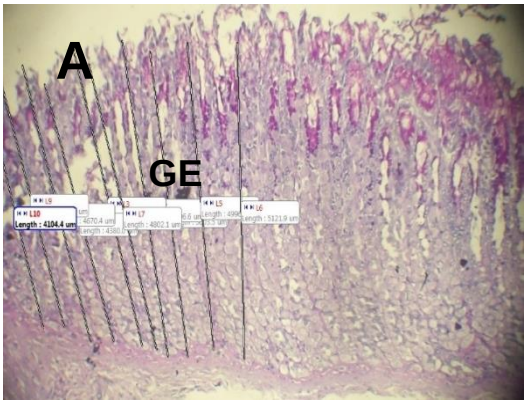
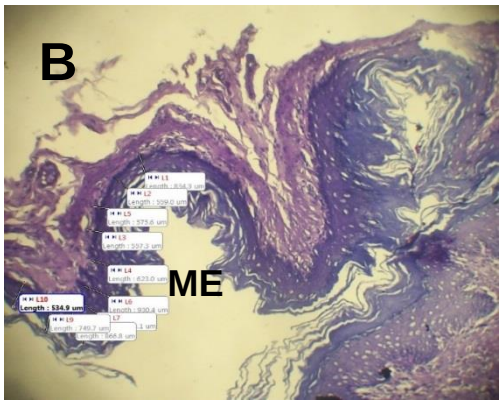
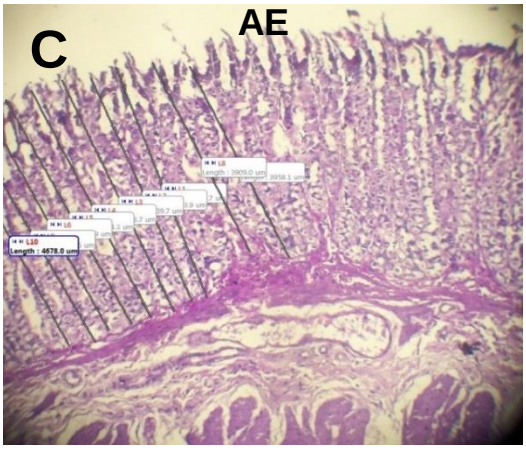
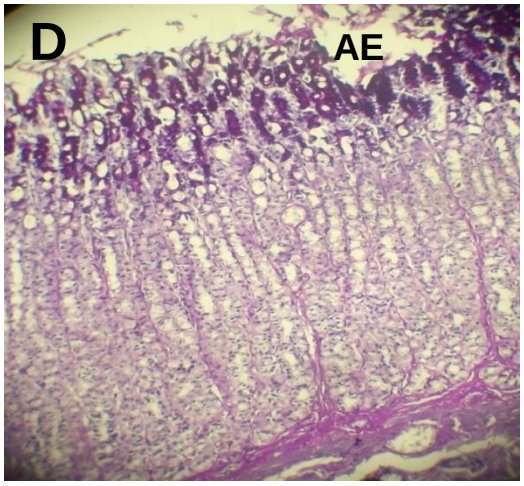
HISTOLOGICAL PROFILE OF THE EFFECTS OF THYROIDECTOMY, THYROXINE REPLACEMENT, OVARIECTOMY, OESTROGEN REPLACEMENT AND TADALAFIL TREATMENTS IN INDOMETHACIN-INDUCED GASTRIC ULCERATION

Histology showed a gastric mucosa disturbance that is in consonance with the pattern of ulceration as evidenced by the various indices of ulceration. Histopathological examination of the gastric mucosa showed a deep alteration of both the glandular epithelium and the submucosa structures as evidenced by widespread apical and glandular erosions as well as epithelia desquamations (FIGURE. 6A, 6B). The loss of histological structures provoked by indomethacin was not clearly evident in the TAD-treated and the thyroxine and oestrogen replaced groups as there were diffuse mucosal erosions with slightly intact glandular portions. Thyroidectomy and ovariectomy worsened ulceration when compared with Control. This is evident from the extensive epithelial exfoliations observed in the tissues (FIGURE 6B, FIGURE 7B). This effect was ameliorated in varying degrees by treatments with tadalafil and in combination with thyroxine and oestrogen respectively resulting in near intact and fairly normal epithelial and glandular structures (FIGURE 6D and E, FIGURE 7D and E).









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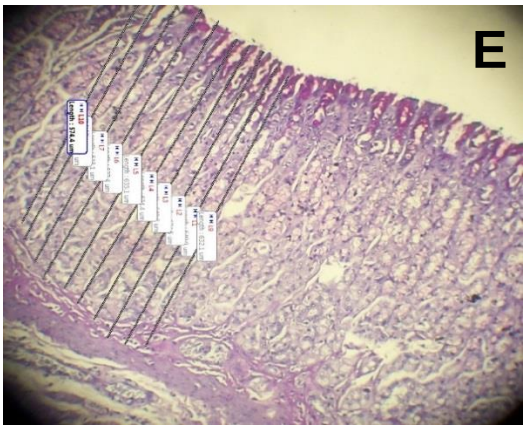


A- Control

B- Thyroidectomised (TMZ)

C- TMZ+TAD 10mg/kg

D- TMZ+TAD10mg/kg+T_4

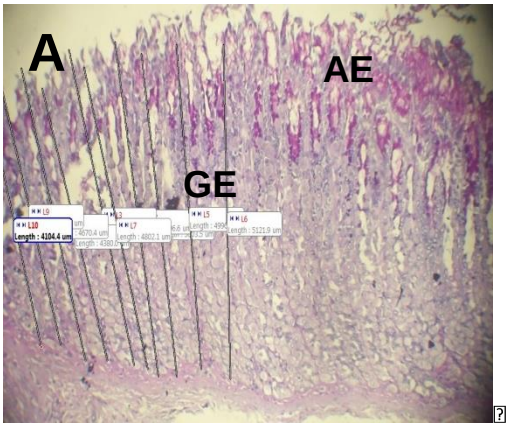
E- TAD 10mg/kg

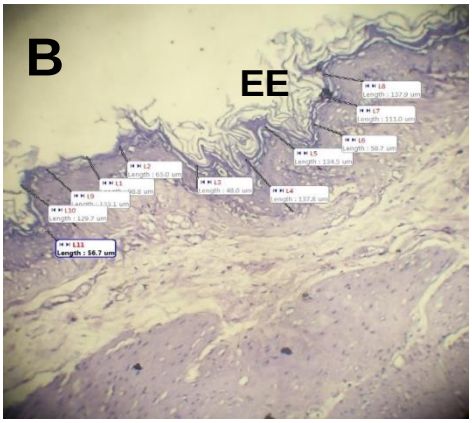
AE- apical erosion

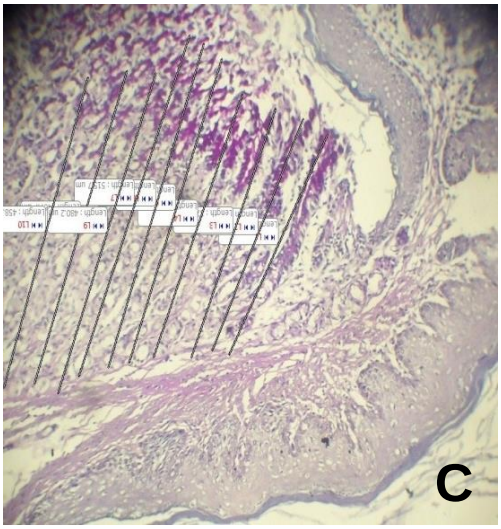
CEM- complete mucosa exfoliation

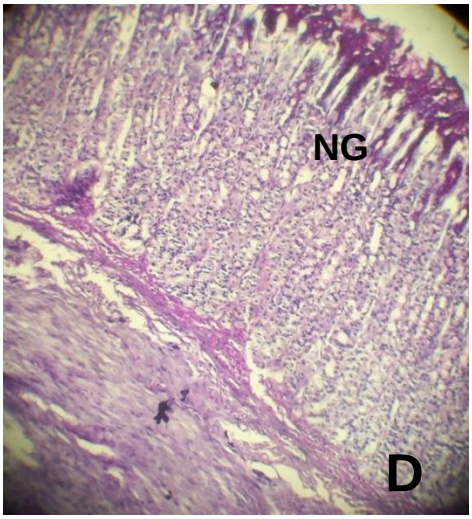
GE- glandular erosion

Fig. 6: Histological profile of the effect of thyroidectomy and thyroxine replacement in indomethacin-induced gastric ulceration. PAS staining. Mag. X100









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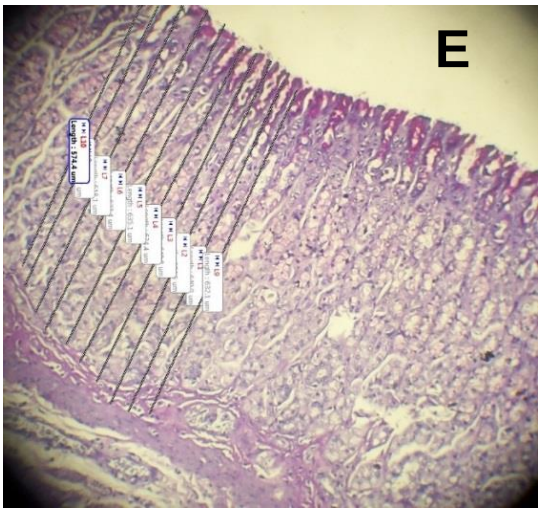

A- Control

B- Ovariectomised (OVMZ)

C- OVMZ+TAD 10mg/kg

D- OVMZ+TAD10mg/kg+OES

E- TAD 10mg/kg

AE- apical erosion

CEM- complete mucosa exfoliation

EE- epithelial exfoliation

NE- normal glands

Fig. 7: Histological profile of the effect of ovariectomy and oestrogen replacement in indomethacin-induced gastric ulceration. PAS staining. Mag. x100

GASTRIC MUCUS PRODUCTION PATTERN IN THYROIDECTOMISED, TADALAFIL AND THYROXINE-TREATED RATS

The pattern of gastric mucus production in response to thyroidectomy, thyroxine replacement and tadalafil treatment in indomethacin-induced gastric ulceration is shown in FIGURE 8. Mucus production is lowered by thyroidectomy. Tadalafil significantly elevated mucus production when compared to control (388±5.8; $p<0.05$). This mucus enhancing effect was diminished in thyroidectomised rats treated with tadalafil (291±3.2). Thyroxine replacement in combination with tadalafil treatment in thyroidectomised rats significantly elevated mucus production when compared to control (410±15.9; $p<0.05$).

Fig 8: Gastric mucus production pattern in thyroidectomised, tadalafil and thyroxine- treated rats.

* - significant at $p<0.05$ when compared with Control
# - significant at $p<0.05$ when compared with TAD only

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GASTRIC MUCUS PRODUCTION PATTERN IN OVARIECTOMISED, TADALAFIL AND OESTROGEN TREATED RATS

The pattern of gastric mucus production in response to ovariectomy, oestrogen replacement and tadalafil treatment in indomethacin-induced gastric ulceration is shown in FIGURE 9. Ovariectomy consistently lowered mucus production when compared to control. There is elevated mucus production in tadalafil-treated rats. Ovariectomised rats treated with tadalafil had a significantly elevated mucus level when compared to control (610±7.1; $p<0.05$). Ovariectomised rats treated with both tadalafil and oestrogen also had significantly elevated mucus content (420±4.9; $p<0.05$).

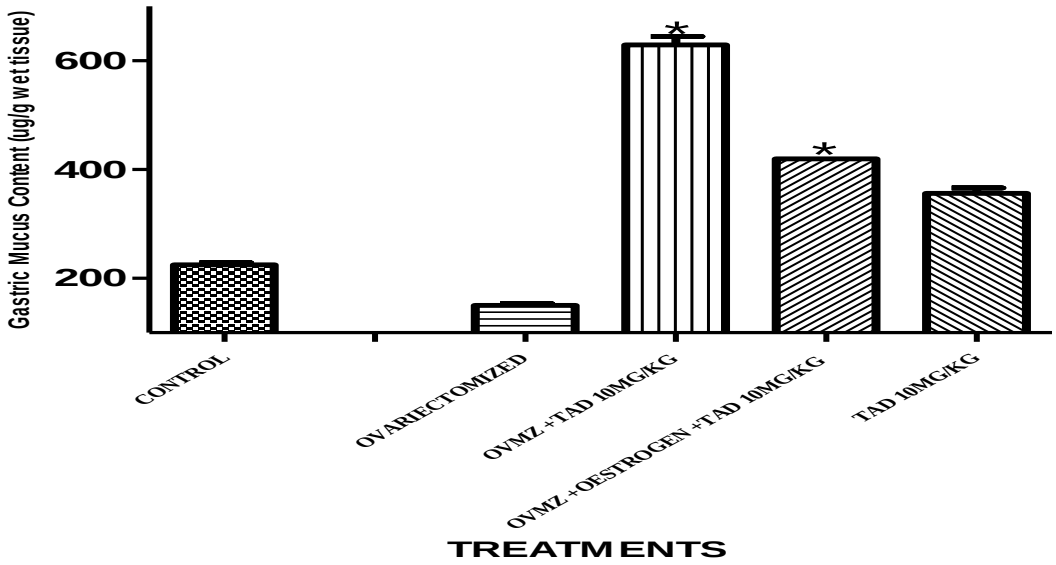

Fig 9: Gastric mucus production pattern in ovariectomised, Tadalafil and oestrogen-treated rats

* - significant at $p<0.05$ when compared with Control

DISCUSSION

Peptic ulcer represents a leading concern among gastrointestinal tract disorders and even though there are a variety of treatment protocols for the condition, each with its varying degree of success and side effects, none has been able to completely 'cure' the condition. Part of the more recent approach directed at tackling the gastric ulcer problem has been focused on improving microcirculation around the ulcerated gastrum. A great deal of focus was and is still being directed at agents that improves blood flow to tissues. One of the most focused on of such agents is nitric oxide and its donors [2], [9]. In our previous study, we established that Tadalafil at high doses, ameliorated the effects of indomethacin-induced ulcerations in rats [10]. However, much of the suggested mechanism of actions was speculative at best. Hence, this study was aimed at investigating the anti-ulcerogenic mechanisms of Tadalafil in indomethacin-induced gastric ulcerations in rats. Furthermore, considering that most users of Tadalafil are the elderly challenged with hormonal imbalances secondary to aging especially the gonadal ones, further investigations were directed at the possible influence if any, the presence or absence of testosterone, oestrogen and thyroxine may have on Tadalafil action in indomethacin-induced gastric ulceration.

The incidence of ulcer has been linked to gender differences up to a certain age. As with several endocrine studies, there are conflicting reports on the effect of oestrogen on ulcer incidence. While some studies have demonstrated the pro-ulcerogenic effects of oestrogen and how it exacerbate NSAID-induced ulcer in rats, others documented its protective effect against same category of agents [11], [12]. In another study, this gastro-protective effect was linked to the duration of use or exposure rather than on dosage [13]. In this study, ovariectomised animals had similar ulcer score compared to Control animals (Table 3). Tadalafil significantly inhibited ulcer formation in

indomethacin-induced animals. However, this inhibitory effect was absent in ovariectomised animals treated with Tadalafil thus suggesting the exacerbating influence of ovariectomy on ulcer formation (FIGURE 3). This correlates with the findings of a study that showed increased ulcer incidence and severity in post-menopausal women [14]. Co-administration of oestrogen and tadalafil to ovariectomised animals significantly lowered the ulcer score thus pointing to a possible potentiation of the tadalafil effect by oestrogen (Table 3). This suggests a potential benefit of tadalafil use in females on oestrogen replacement therapy.

There is a strong correlation between the observed ulcer score and the ulcer dimensions (area and depth). Significant reductions in the ulcer dimensions were observed in animals treated with Tadalafil only and those co-administered with oestrogen and Tadalafil when compared with Control (Table 6). The ovariectomised animals had a severely compromised tissue integrity resulting in increase in ulcer dimensions. Oestrogen has also been shown to play major roles in the maintenance of tissue integrity by increasing the number of tight junctions in tissues. These tight junctions are found to decrease drastically in menopause [15], [16]. These effects of oestrogen are mediated via oestrogen receptors alpha and beta (ERα and ERβ) both of which are expressed in a variety of cells such as muscles, immune and epithelial cells. Therefore, decreased oestrogen production in aging has been directly linked with negatively impacting on mucosal health and recovery [17]. Furthermore, in addition to the ERα and ERβ mediated oestrogenic activity, it has been shown that direct antioxidant mechanisms in anti-inflammatory function limiting neutrophil movement to damaged tissue thus reducing inflammatory cytokine production and alleviating tissue damage [18]. More males have been shown to develop ulcer up to the age of 70 years after which the gender effect seem to pan out and make no significant difference [14]. This observation has also been linked to the presence of oestrogen in the females and an increase in the parietal cells in the males [19]. Studies have also reported increased gastric and intestinal tissue resistance secondary to oestrogen administration [20].

There is a strong connection between changes in the male hormone testosterone and gastric ulcer. Several studies have highlighted both the pro and anti-ulcer effects of the physiological presence or absence of testosterone on gastric ulcer formation. While it has been shown that tadalafil significantly lowered the ulcer score of indomethacin treated rats, this effect was however not observed in the castrated rats administered with Tadalafil (Table 2). Tadalafil significantly reduced the ulcer depth in both the castrated and the uncastrated rats (Table 5). Ulcer scoring was higher in castrated rats when compared to Control (although not statistically significant), while testosterone supplementation with Tadalafil produced no inhibitory effect on ulcerogenesis (FIGURE 2). Contrary to reports of some studies which showed the increased susceptibility of the gastric mucosa to injury secondary to testosterone presence [21], [22], [23] and others that highlighted the beneficial effects of testosterone in protecting against NSAID-induced ulcers [24], the findings of this study showed no significant difference on the effect of testosterone withdrawal and/or supplementation on ulcer formation. There are similar dimensions of ulcerations both in area and depth between the Control and the testosterone administered rats, thus neither aggravating nor diminishing the indomethacin effects on the gastric mucosa. It may therefore be safely concluded that testosterone has no significant influence on tadalafil action on ulcerogenesis in indomethacin treated rats.

The relationship between the hormones of the thyroid gland (tri-iodothyronine and thyroxine) and ulcer formation remains a keenly debated and continuously researched issue. Hyperthyroidism has been demonstrated to have a certain protective effect in relation to ulcer formation with T_3 and T_4 administration effectively reducing the length and depth of ulcer formation especially in the stress ulcer induction model [25]. Furthermore, hypothyroidism was associated with increased stress-related gastritis [26]. In the current study, the ulcer score was elevated by thyroidectomy while tadalafil acting alone significantly reduced the ulcer score. Thyroxine replacement in tadalafil treated rats produced a greater inhibition of gastric ulcer formation compared to control (Table 1). This suggests possible modulation of the activity of tadalafil by thyroxine. It is reported that thyroxine reduced the degree of ulceration in rats by a mechanism that facilitates rapid production of gastric mucus [27]. The percentage inhibition of ulcer was significantly higher in the thyroxine+tadalafil supplemented rats. This result buttresses the outcome of the preliminary study in which it was established that tadalafil possesses anti-gastric ulcer properties [10].

Ulcer formation has been directly linked to factors such as gastric volume, free and total acidity and histamine secretion [28], [29]. The mechanism of action for histamine is usually either via the activation of histamine receptors (H_2 receptors) found on parietal cells which are responsible for acid secretion or via an increase in cAMP [30]. While there have been evidences of NO and NO-donors like sodium nitro-prusside being involved in gastric acid secretion mediated by histamine from acid-secreting enterochromaffin-like cells (ECL), other authors have reported an inhibitory role for the NO-cGMP moiety in the process of gastric acid secretion including histamine release from ECL cells [31], [32]. In our preliminary study, Tadalafil failed to potentiate the action of histamine on both the basal and stimulated gastric acid secretion [10]. The finding from the current study is consistent with this observation.

There were significant reductions in the level of histamine activity and potentiation between the control and when tadalafil was co-administered with oestrogen (FIGURE 5). Histamine is released from ECL cells in response to gastrin stimulation. Histamine release in turn signal parietal cells to produce hydrochloric acid via H_2 receptors. One of the earliest reports on oestrogen influence on ulcer established that ovariectomised rats treated with oestrogen (17-β oestradiol) showed a statistically significant reduction in gastric acid secretion [33]. They concluded that elevated plasma oestrogen level is capable of inhibiting gastric acid secretion. Observation from the current study is consistent with this report. There is a potentiation of histamine action in both the control and the ovariectomised rats and a significant reduction in acid secretion when oestrogen was administered to tadalafil treated rats suggesting an anti-histaminic effect of oestrogen. Another mechanism by which oestrogen may be exerting anti-secretory effects may be via a cholinergic action. An inverse relationship was noted in a study comparing gastric acid output with plasma oestrogen concentrations in women. This effect was blocked by atrophine sulphate administration thus suggesting a cholinergic action in play [35].

Evidences from several studies conducted on both humans and animals had concluded that thyroid hormone is inhibitory to HCl production by the stomach [35],[36]. However, data from this work showed a significant increase in acid secretion in response to thyroxine administration (Table 4). This action was not suppressed by co-administration with Tadalafil or by thyroidectomy although the free acidity level remained unchanged. This result is in line with the school of thought that supports the theory of an overall rise in gastric acid secretion following thyroxine administration. There is argument argued against decreased gastric acid secretion in hypothyroid rats but showed that secreted volume, acid concentration and output were all increased secondary to thyroxine administration [37]. One of the earliest reports on histamine influence on gastric secretion later showed that there is increased possibility of acid hyper-secretion in response to thyroid hormone administration [38]. The study reported that 50% of hyperthyroid patients studied had elevated gastric secretory activity. This was supported by more recent works that showed elevated basal and stimulated acid secretion in rats [36]. The mechanism underlying these observed effects is still largely unknown, but plausible explanations proposed by [21] and [36] included that thyroxine increases the total number of parietal cells in the stomach thus leading to increased HCl production and that thyroid hormones exert their influence on the number and/or size of the secretory cells in the stomach. Further test may be needed to ascertain if the increased acid secretion produced in response to thyroxine administration is as a result of potentiation of histamine action or it is due to an increase in the number or size of the acid secretory cells. The histopathologic results of the various sections (FIGURE. 6–7) showed some remarkable differences in the stomach tissues. There is a deep alteration of glandular epithelium and a loss of histological structures consistent with the characteristic effects of indomethacin action on the gastric mucosa. Although the actions of NSAIDs on the gastric epithelium may involve several mechanisms, most NSAIDs, particularly those of acidic nature, can directly kill or denature epithelial cells leading to necrosis and epithelial exfoliation [39]. The differences observed in the degree of damage across the various treatment groups are likely attributable to the effects of the surgical operations (thyroidectomy and ovariectomy) and the replacement therapies employed. Tadalafil demonstrated significant cyto-protective effects as evidenced by an almost intact mucosa epithelium (FIGURE. 6E, FIGURE. 7E). Thyroidectomy (FIGURE. 6B) and ovariectomy (FIGURE. 7B) worsened ulceration when compared with control (FIGURE. 6A, FIGURE. 7A). This effect was ameliorated in varying degrees by treatments with tadalafil alone (FIGURE. 6C, FIGURE. 7C) and in combination with thyroxine and oestrogen (FIGURE. 6D and FIGURE. 7D respectively). These observations are consistent with the pattern of ulcer score and dimension.

The estimated gastric wall mucus has been used as an index for gastric mucus secretion [40]. There is evidence of correlation between the protection afforded against experimental ulcers and mucus production. The stomach is protected by a layer of mucoid secretion which serves both as lubricant and as a protective barrier between the surface of the gastric mucosa and the luminal contents. This mucoid layer is part of the gastric mucosal barrier described by [41] as being integral to the stomach's ability to inhibit the diffusion of H^+ from the luminal side into the mucosa and diffusion of Na^+ in the reverse direction. [42] put forward a theory that the mechanism of NSAID-induced gastric damage may be via reduced secretion of gastric mucus. We tested the theory of [43] that NO and its donors, such as Tadalafil, may protect against NSAID-induced gastric damage by the promotion of prostaglandin and or mucus synthesis. In our preliminary results, we established that Tadalafil acting alone significantly increased mucus production in a pattern similar to the effect of prostaglandin analogue, misoprostol [10].

Tadalafil significantly increased mucus secretion when compared to control (FIGURE 8). A similar significant elevation in mucus production was observed in ovariectomised rats treated with tadalafil (FIGURE. 9). There was a significant increase in mucus production when tadalafil was co-administered with oestrogen signifying a potentiation of tadalafil action by oestrogen replacement in ovariectomised rats. [19] reported decreased mucus and phospholipid levels in ovariectomised rats. [44] however reported a compensatory mechanism where in oestrogen deficiency led to increased gastric mucosa synthase activity which mediates mucus overproduction in ovariectomised rats, thus protecting against the damaging effects of ovariectomy on the gastric mucosa. It may be concluded therefore, that oestrogen acting in concert with tadalafil, increased the mucus secreting capability of the gastric mucosa.

The connection between thyroxine and gastric mucus production has been an unresolved debate as various contributors over the years have fallen on either side of the 'for or against' argument. This is not unrelated to thyroxine's role as an enhancer of secretagogue action on gastric acid secretion. Co-administration of tadalafil and thyroxine resulted in significant production of mucus in thyroidectomised rats (FIGURE. 8). Increased and rapid mucus production secondary to thyroxine treatment in rats has also been reported in some studies [27]. While the mechanism involved remains unclear, it has been suggested that potentiation of prostaglandin synthesis may have been involved. The presence of the mucoid cap is essential for the maintenance of epithelial permeability which prevents gastric acid back diffusion [45]. Thyroxine modulates the mucoid cap by not just the increasing the number of cells that produce both neutral and acidic secretions but also via an increase in the total expression of their mucins in indomethacin-induced ulcers.

CONCLUSION

This study was designed to investigate the anti-secretory and mucogenic effects of the sex hormones and thyroxine on ulcerogenesis in tadalafil-treated Wistar rats. Mean ulcer score and ulcer area were significantly reduced by tadalafil and the combination of tadalafil and either of oestrogen or thyroxine. Castration and testosterone replacement did not have any significant effect on gastric ulceration. Total titratable and stimulated acid secretions were significantly decreased by tadalafil and oestrogen. This anti-secretory and mucogenic gastro-protective effects is enhanced by thyroxine and oestrogen, but not by testosterone in Wistar rats.

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