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# Journal of Physiology and Pathophysiology

Table of Content:      Volume 10    Number 1    May 2019

## ARTICLES

- Carpolobia lutea methanol root extract reinstates androgenesis and testicular function in cadmium-challenged rats** **1**  
Ejike Daniel Eze, Okpa Precious Nwaka, Igbokwe Ugochukwu Vincent,  
Moses Dele Adams, Karimah Mohammed Rabiu and Ayikobua Emmanuel Tiyo
- Evaluation of the cytoprotective effects of anti-ulcer agents in acid-alcohol induced gastric ulceration in wistar rats** **10**  
Nworgu Choice, Celestine Ani, Ugwuishi Emeka, Okorie Pamela, Anyaeji Pamela,  
Ugwu Princewill, Uzoigwe Jide, Igwe Uzoma and Nwachukwu Daniel

*Full Length Research Paper*

# ***Carpolobia lutea* methanol root extract reinstates androgenesis and testicular function in cadmium-challenged rats**

**Ejike Daniel Eze<sup>1,2\*</sup>, Okpa Precious Nwaka<sup>3</sup>, Igbokwe Ugochukwu Vincent<sup>3</sup>,  
Moses Dele Adams<sup>4</sup>, Karimah Mohammed Rabiu<sup>5</sup> and Ayikobua Emmanuel Tiyo<sup>2</sup>**

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Cadmium produces a wide range of biological dysfunctions in human and laboratory animals where it chiefly affects the testes, whereas, *Carpolobia lutea* has been known to have antioxidant benefits. This study was intended to investigate the effects of *Carpolobia lutea* root extracts on testicular hormone function in cadmium-challenged male rats. 48 male Wistar rats (170-190 g) were divided into six groups, each containing eight rats. Experimental animals in control (Group 1) were given 0.2ml/kg body weight (BW) of 10% tween 80; Group two were administered 1mg/kg BW of cadmium (i.p); Group 3 were given 1mg/kg BW of cadmium (i.p) + 100mg/kg BW extract; Group four took 1mg/kg BW of cadmium (i.p) + 200mg/kg BW extract while Group five and six got 100mg/kg and 200mg/kg BW extract respectively. The administration of vehicle and extract was conducted orally for six weeks. Testicular activity of 17 beta-hydrosteroid dehydrogenase (17 $\beta$ -HSD) and serum testosterone, luteinizing and follicle stimulating hormone (LH, FSH) levels were evaluated. Findings indicated that cadmium statistically ( $p < 0.05$ ) lowered testicular 17 $\beta$ -HSD activity and serum testosterone, LH and FSH levels when compared with those of the control group animals. However, *Carpolobia lutea* and its co-administration notably ( $p < 0.05$ ) elevated the activity of testicular 17 $\beta$ -HSD and levels of serum testosterone, LH and FSH. The study suggests that *Carpolobia lutea* extract plays a protective function in ameliorating testicular damage caused by cadmium in rats. This is probably due to the extract's potential in the management of testicular dysfunction and fecundity in animals.

**Key words:** *Carpolobia lutea*, polygalaceae, cadmium, 17 beta-hydrosteroid dehydrogenase, testicular function.

## **INTRODUCTION**

Life is scheduled by nature from the least microbe to the highest whale to have one basic quest, which is to

recreate. In addition to other required human activities, reproduction is its basic goal (Taflinger, 1996).

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Reproduction is the biological process by which new organisms are produced from their parents. The irony of this is that some people still find it difficult to procreate as a result of infertility. Infertility is defined as the inability of sexually active persons to achieve conception despite regular unprotected sexual activity for a period of one year (Belsey, 1976; Yales et al., 1989; Bonde, 1996; Yesilli et al., 2005). Infertility in men is germane because they determine conception and sex (gender). One of the causes of male infertility is exposure to environmental toxicant. Others are chemotherapy, hypothalamic-pituitary factors, pre-testicular factors, isolated incidences of deficiency in gonadotropin, luteinizing hormone and follicle stimulating hormone as well as androgen excess, glucocorticoid excess and hyperprolactinemia (Reijo et al., 1996; Rosendahl et al., 2010; Olooto et al., 2012).

Cadmium (Cd) is one of the heavy metals and a potent environmental toxicant. Humans are exposed to Cd via contaminants found in drinking water and food; occupational exposition during mining and manufacturing of batteries and pigments that contain Cd; industrial activities such as smelting and refining of metals and municipal waste incineration which release Cd into the atmosphere as well as inhaling tobacco smoke (Blanco et al., 2007; Siu et al., 2009). Cd toxicity is associated with severe damage to testes of both human and animals (Fouad et al., 2009).

Rodent testes are especially sensitive to the deleterious effects of Cd exposure. This is because Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats (Burukoglu and Baycu, 2008). The adverse consequences of exposure to Cd on the reproductive organs have been widely considered. Serum levels of testosterone, LH and FSH were notably lowered in the Cd exposed rats (Sadik, 2008).

The reduced activity or privation of male hormones to the testes by Cd has been reported to adversely affect the normal functioning ability of the testes and consequently male fertility (Gupta et al., 2004). Therefore, this growing evidence of toxicity to testes needs to be addressed by screening natural products with claimed androgenic, restorative or ameliorative potentials (Farnsworth, 1989; Eisner, 1990).

*Carpolobia lutea* G. Don (Family: Polygalaceae), a small tree growing up to 15ft in height (Akpan et al., 2012) is wide spread across the west and central areas of tropical Africa (Mitaine-offer et al., 2002; Nwidiu et al., 2015). The common names include cattle stick (English); ikpafum, ndiyan, nyayanga (Ibibio); abekpok ibuhu (Eket); agba, angalagala (Igbo); egbo oshunshun (Yoruba); (Kayode and (Kayode and Omotoyinbo, 2008; Ogunwande et al., 2014). The plant has been scientifically validated to have the following medicinal effects: analgesic (Ajibesin et al., 2008, Jackson et al., 2011), protection of the GIT (Nwidiu and Nwafor, 2009), anti-diarrheal (Nwidiu et al., 2014), anti-diarrhoeal and

treatment of ulcer (Nwafor and Bassey, 2007), anti-microbial (Etebong and Nwafor, 2009), aphrodisiac (Mitaine-offer et al., 2002), antimalarial (Okokon et al., 2011), contraceptive (Etebong et al., 2011), antidiabetic and hypolipidemic (Akpan et al., 2012), acute and sub-acute toxicity (Nwidiu et al., 2012), antiacetylcholinesterase activity and antioxidant.

Dare et al. (2015) reported that combined administration of *C. lutea* methanol root extract at 40 and 80 mg/kg body weight and sildenafil attenuated reproductive function impairment in male rabbit while Yakubu and Jimoh (2015) saw that 47, 94 and 141 mg/kg body weight of *C. lutea* aqueous root extract reinstated testicular function and sexual capacity in paroxetine-challenged male rats. Despite these streams of fertility findings, there is need to carry out scientific investigation on the male sexual ability of the plant at ethnobotanically acclaimed doses of 100 and 200 mg/kg body weight on toxicity caused by cadmium in rats. Therefore, this study seeks to evaluate the androgenic and testicular effect of methanol extract of *C. lutea* root on cadmium-challenged rats.

## METHODOLOGY

### Authentication of plant material

The roots of *C. lutea* were obtained from Ijare, a village at Akure, Ondo state and authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a Voucher number 109784 was designated to the specimen of *C. lutea*.

### Animal model

Forty eight adult male Wistar rats were obtained from the Animal Care Unit of Igbinedion University, Okada, Edo State. Animals weighed between 170 and 190 g and were housed in a well-ventilated plastic cage and maintained under standard laboratory condition. They ate standard commercial rat pellets and drank clean water daily. Ethical guidelines for animal use were approved by the Institutional Animal Ethics Committee of the Igbinedion University, Okada, Edo State, Nigeria.

### Extract preparation

The *C. lutea* root was air dried and pulverized. 2000 gram of the pulverized powder was soaked in 5 litres of methanol for 48 h and filtered. The filtrate was then concentrated using a freeze-drier. The extract was thereafter dried in a vacuum oven at 40° for 3 h to ensure the removal of leftover solvent. This yielded 50.29 g of semi-solid extract (2.51% yields) was then stored in a refrigerator for the study. The extract was re-diluted in 10% Tween 80.

### Experimental design, animal grouping and treatment

The rats were acclimatized for two weeks. There are 6 groups of eight rats each and treated as follows (Table 1). The animals were treated with vehicle, cadmium (i.p single dose) and *Carpolobia lutea* methanol roots extract orally for 6 weeks. 24 h after the last

**Table 1.** Animal grouping and treatment.

Experimental group	Treatment
I (Control)	0.2 ml/kg BW (10% tween 80 in solution)
II	1 mg/kg BW cadmium ( <i>i.p</i> single dose)
III	1 mg/kg BW Cd ( <i>i.p</i> single dose) + 100 mg/kg BW methanol root extract of <i>C. lutea</i>
IV	1 mg/kg BW Cd ( <i>i.p</i> single dose) + 200 mg/kg BW extract
V	100 mg/kg BW extract
VI	200 mg/kg BW extract

Number of animals per group =8.

administration, the animals were sacrificed; blood was collected from the animals via cardiac puncture for hormonal assay while the testes collected was weighed and used for the determination of 17  $\beta$ -HSD activity.

#### Determination of serum testosterone

Serum testosterone (17 $\beta$ -hydroxyandrost-4-ene-3-one) level was determined by adopting the procedure described by Chen et al. (1991). Briefly, upon getting the desired number of coated wells, 10  $\mu$ l of standards, specimens and controls was poured into appropriate wells. 100  $\mu$ l of testosterone-horseradish peroxidase (Testosterone-HRP) Conjugate Reagent was added into each well. This was followed by topping each well with 50  $\mu$ l of rabbit anti-Testosterone reagent and thoroughly mixed for 15 s. The mixture was warm at 37°C for 70 min after which the incubation mixture was removed by flicking plate contents into a waste container. The microtiter wells were rinsed and flicked 7 times with deionized water. The wells were then shaken sharply onto absorbent paper to remove all residual water droplets. Thereafter, 100  $\mu$ l of tetramethylbenzidine (TMB) Reagent was pipetted into each well, mixed for 5 s and kept at room temperature for 15 min. 100  $\mu$ l of Stop Solution was added to each well to stop the reaction. It was mixed gently for 25 s to allow blue color changes to yellow color completely. Absorbance was then read at 450 nm with a microtiter well reader within 10 min.

#### Computation of results obtained

The mean optical density value (OD<sub>450</sub>) was computed for each set of reference standard, control and sample. A standard curve was drawn by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a straight line graph paper; it has absorbance values on the vertical (y) axis, and concentrations on the horizontal (x) axis. Using the mean absorbance values for each sample, the corresponding concentration of testosterone in ng/ml was determined from the calibration curve. Values obtained for diluted samples were further converted by applying the appropriate dilution factor in the calculations.

**Principle:** The Testosterone ELISA follows the principle that competitive binding between testosterone in the test sample and testosterone-horseradish peroxidase (HRP) conjugates for a constant amount of rabbit anti-Testosterone. During the warming process, goat anti-rabbit IgG-coated wells are warmed with testosterone standards, controls, patient samples, testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent for a period of 60 min. During the incubation, a fixed amount of HRP-labeled testosterone competes with the testosterone in the

standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the quantity of testosterone-HRP immunologically bound to the well gradually decreases as the concentration of testosterone in the rat sample increases. Unbound testosterone-peroxidase conjugate is then removed and the wells washed, followed by addition of TMB Reagent resulting in the development of blue color. The color development is stopped and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color produced is directly-related to the amount of enzyme present and is conversely-related to the amount of unlabeled testosterone in the sample. A calibration curve is obtained by plotting the concentration of the standard against the absorbance. The testosterone concentration of the test sample and controls run concurrently with the standards can be calculated from the standard curve.

#### Determination of serum luteinizing hormone

Serum luteinizing hormone was analysed by adopting the procedure described by Nielsen et al. (2001). Briefly, to the coated wells arranged in a rack, 50  $\mu$ l of standards, test samples, and controls was placed rightly in labelled wells. 100  $\mu$ l of Enzyme Conjugate Reagent was added into each well, mixed slowly for 20 s and left at room temperature (18-25°C) for 30 min. The incubation mixture was removed by shaking the plate contents into sink. The mixture was cleaned with water and the microtiter wells were stirred 5 times with distilled water. The wells were placed immediately onto paper towels to eliminate all residual water droplets. This was followed by adding 100  $\mu$ l of tetramethylbenzidine (TMB) Reagent into each well and mixing gently for 10 s. The mixture was left at room temperature for 30 min after which 100  $\mu$ l Stop Solution was added to each well to stop the reaction. It was slowly mixed for 20 s to ensure that all of the blue color changes completely to yellow color. Absorbance was thereafter read at 450 nm with a microtiter plate reader within 10 min.

#### Calculation of results

Mean absorbance value (A<sub>450</sub>) was computed for each set of reference standards, controls and test samples. The corresponding concentration of LH in mIU/ml from the standard curve was determined using the mean absorbance value for each test sample.

**Principle:** Enzyme immunoassay test follows a typical two-step approach or 'sandwich' type assay. The assay utilizes two highly specific monoclonal antibodies: A monoclonal antibody specific for LH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of LH is conjugated to horse radish peroxidase (HRP). LH from the sample and standards are allowed to bind to the plate, washed, and warmed thereafter with



the HRP conjugate. After a second washing step, the enzyme substrate was added. The enzymatic reaction was stopped by addition of the stopping solution. The absorbance was measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample. A set of standards was used to plot a calibration curve from which the amount (concentration) of LH in the test samples and controls can be directly read.

#### Determination of serum follicle stimulating hormone

Serum follicle stimulating hormone was determined by using the protocol stipulated by Butt et al. (1973). Briefly, the followings were prepared: work solutions of the anti-FSH-HRP conjugate, wash buffer and the required number of well strips. 20  $\mu$ l of each calibrator, control and test sample was added into respectively marked wells in duplicate followed by addition of 80  $\mu$ l of assay buffer into each well. This was heated on a plate shaker (exactly 300 rpm) for 20 min at 25°C. The wells were washed 3 times with 300  $\mu$ l of diluted wash buffer per well. This was followed by adding 90  $\mu$ l of the conjugate work solution into each well after which it was warmed on a plate shaker (exactly 150 rpm) for 15 min at 25°C and the wells were washed again as earlier done. About 70  $\mu$ l of TMB substrate was put into each well at 5 min interval. The mixture was heated on a plate shaker for 20 min at 25°C (or until calibrator F attains dark blue color for desired OD). About 30  $\mu$ l of stop solution was added into each well at 5 min interval after which the plate was spectrophotometrically read on a microplate reader at 450 nm within 10 min after adding of the stop solution.

#### Calculations

The mean absorbance of each unknown duplicate was computed by minusing the average optical density value of the "0" calibrator from the mean absorbance values of the calibrators, controls and test serum samples. A calibrator curve was drawn on log-log sheet of paper with the average optical densities on the Y-axis and the calibrator concentrations on the X-axis. The unknown values were read directly off the calibrator curve at a standard wavelength.

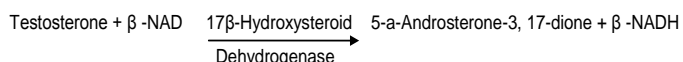
**Principle:** The principle follows a typical two-step strategy or 'sandwich' type assay. The assay employs two highly potent monoclonal antibodies: a monoclonal antibody specific for FSH is immobilized onto the microplate and another monoclonal antibody active for a different region of FSH is conjugated to horse radish peroxidase (HRP). FSH from the test sample and standards are allowed to bind to the plate, washed, and thereafter warmed at room temperature with the HRP conjugate. After a second washing step, the enzyme substrate was added. Addition of the stop solution terminated the enzymatic reaction. The absorbance was measured on a microtiter plate reader. The degree of the color formed by the enzymatic reaction was directly-related to the concentration of FSH in the test sample. A set of standards was used to plot a calibration curve from which the amount of FSH in rat test samples and controls can be spectrophotometrically read.

#### Determination of testicular 17 beta-hydrosteroid dehydrogenase (17 $\beta$ -HSD) [EC 1.1.1.51] activity

Harvested testes were homogenized in 2 ml of normal saline. The homogenate was centrifuged at 1,000 rpm for 15 min. The supernatant collected was employed for the estimation of 17 $\beta$ -HSD. The activity of testicular 17 $\beta$ -HSD was measured according to Jarabak et al. (1996). Briefly, 1 ml of the supernatant was mixed with 1 ml of 440  $\mu$ mol sodium pyrophosphate buffer (pH 10.2), 40  $\mu$ l

of ethanol containing 0.3  $\mu$ mol of testosterone (Sigma) and 960  $\mu$ l of 25% BSA (Sigma), making the incubation mixture a total of 3 ml. The enzyme activity was measured after addition of 1:1  $\mu$ mol NAD (Sigma) to the tissue supernatant mixture in a U2000 spectrophotometer cuvette at 340 nm against a blank (without NAD). 1 unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

**Principle:** The highly specific antibodies pre-coated 96-well microtiter plate containing samples, blanks and standards, incubated with a highly specific biotin-conjugated primary antibody and Avidin conjugated to Horseradish Peroxidase (HRP) and incubated for 10 minutes. After washing the plate and addition of the TMB (3,3',5,5'-Tetramethylbenzidine) solution, the appearance of a blue colour should be detected due to an enzymatic reaction catalysed by HRP. Aspiration of Stop Solution terminates the HRP reaction causing a colour change from the blue to yellow with the signal intensity read spectrophotometrically at 450 nm on a plate reader. The amount of bound 17-beta-hydroxysteroid dehydrogenase is proportional to the signal generated by the reaction.



#### Data presentation and analysis

Findings derived from this work were expressed as mean  $\pm$  SEM of eight determinations. Readings obtained were statistically analyzed using ANOVA and Duncan Multiple Range Test and difference between mean were taken to be substantive at  $p \leq 0.05$ . Significant difference was determined by analysis of variance and Duncan Multiple Range Test at 5% confidence level using SPSS 23.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

## RESULTS

Testicular 17 $\beta$ -HSD activity was substantively ( $p < 0.05$ ) decreased in cadmium when placed with the distilled water treated control group, but was elevated in cadmium plus low dose extract, cadmium plus high dose extract and low dose extract treated rats when matched with cadmium treated animals (Table 2 and Figure 1).

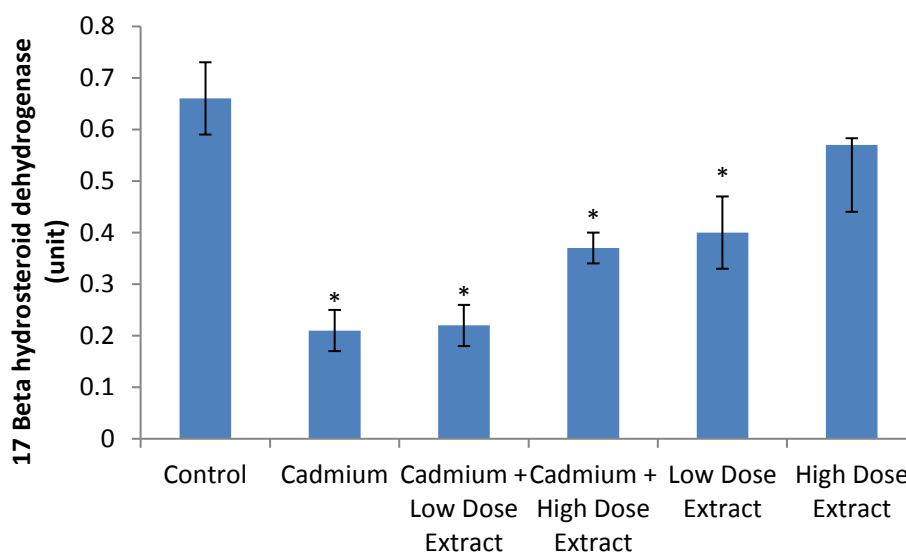
The result showed that LH was significantly ( $p < 0.05$ ) decreased in cadmium, cadmium plus low dose extract treated rats when compared with control group animals. Also, serum LH level was appreciably ( $p < 0.05$ ) elevated in high dose extract and low dose extract treated rats when placed side by side with control group animals ( $p < 0.05$ ). Serum LH concentration was notably ( $p < 0.05$ ) increased in cadmium plus high dose extract treated rats when weighed up with cadmium treated animals (Figure 2).

The result showed that serum FSH was sufficiently ( $p < 0.05$ ) decreased in cadmium, cadmium plus low dose treated rats when set side by side with the control group animals. Also, serum FSH was notably ( $p < 0.05$ ) increased in high dose extract treated rats when measured with control groups. Serum FSH was remarkably ( $p < 0.05$ )

**Table 2.** Methanol root extract of *C. lutea* on 17 $\beta$ -HSD, LH, FSH and testosterone in cadmium-induced testicular toxicity in male Wistar rats.

Group	17 $\beta$ HSD (unit)	LH (m/ $\mu$ /ml)	FSH (m/ $\mu$ /ml)	Testosterone (ng/ml)
Control	0.66 $\pm$ 0.07	16.51 $\pm$ 0.96	6.40 $\pm$ 0.42	8.62 $\pm$ 0.58
Cadmium	0.21 $\pm$ 0.04*	5.90 $\pm$ 0.76*	3.11 $\pm$ 0.47*	3.81 $\pm$ 0.52*
Cadmium + low dose extract	0.22 $\pm$ 0.04*	7.72 $\pm$ 1.28*	4.04 $\pm$ 0.23*	5.54 $\pm$ 0.58*
Cadmium + high dose extract	0.37 $\pm$ 0.03*	13.24 $\pm$ 1.18+	7.36 $\pm$ 0.48+	8.46 $\pm$ 0.86+
Low dose extract	0.40 $\pm$ 0.07*	21.95 $\pm$ 1.77*	7.80 $\pm$ 0.76	8.35 $\pm$ 0.47
High dose extract	0.57 $\pm$ 0.13	28.45 $\pm$ 0.56*	12.52 $\pm$ 1.26*	11.82 $\pm$ 1.01*

\*+ shows significant difference of  $p < 0.05$  when compared with control group and cadmium groups respectively. Low dose extract = 100 mg/kg body weight; High dose extract = 200 mg/kg body weight.



**Figure 1.** Methanol root extract of *C. lutea* on 17 $\beta$  HSD in cadmium- induced testicular toxicity in male Wistar rats. \*+ shows significant difference of  $p < 0.05$  when compared with control group and cadmium groups respectively. Low dose extract = 100 mg/kg body weight; High dose extract = 200 mg/kg body weight.

increased in high dose extract treated rats when juxtaposed with cadmium treated group (Figure 3).

The figure shows that serum testosterone was glaringly ( $p < 0.05$ ) reduced in cadmium, cadmium plus low dose treated rats when likened with the distilled water treated control group animals. Also, serum testosterone was exceptionally ( $p < 0.05$ ) increased in high dose extract treated rats in comparison with rats in the control group ( $p < 0.05$ ). Serum testosterone was meaningfully ( $p < 0.05$ ) decreased in cadmium plus high dose extract treated rats when related with cadmium treated animals (Figure 4).

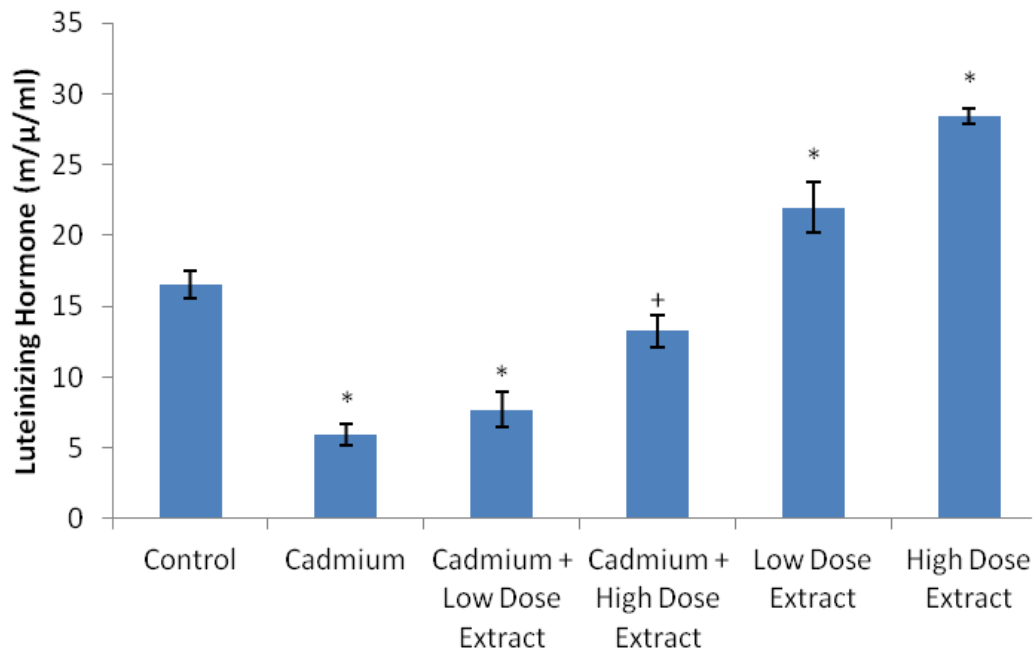
## DISCUSSION

Cadmium produces a wide range of physiological damages in experimental animals and clinical models, affecting mainly the testes (Oteiza et al., 1999). The

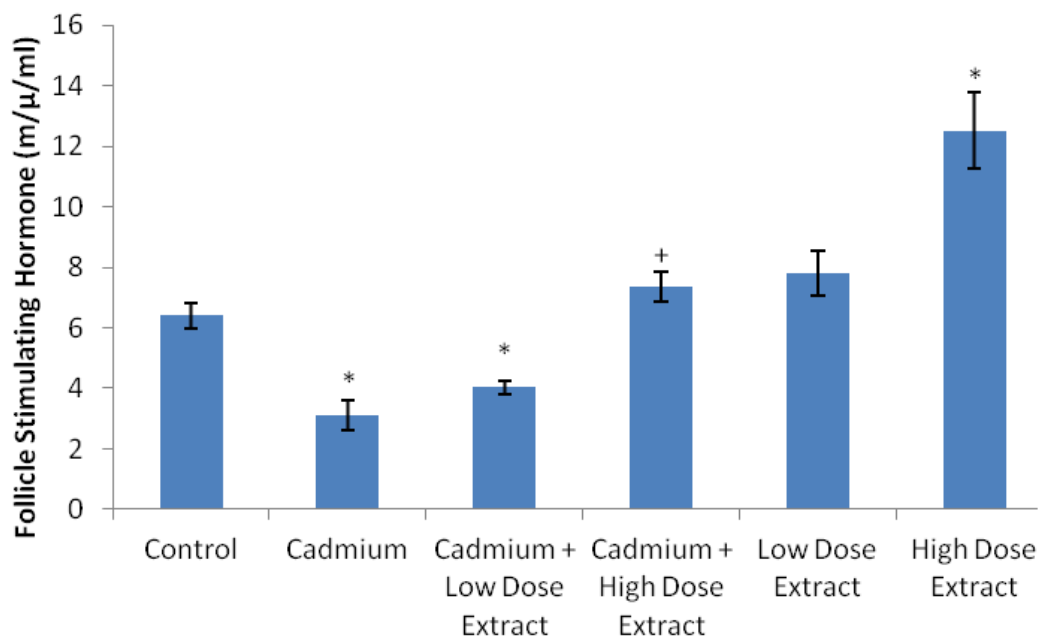
present study intends to examine the effects of methanol root extract of *C. lutea* on 17 $\beta$ -HSD, LH, FSH and testosterone in cadmium-induced damage on testes of Wistar rats.

Information gathered from the present work revealed that exposure to cadmium could affect male fertility. The levels of serum testosterone, luteinizing hormone and follicle stimulating hormone were meaningfully lowered in the cadmium treated rats as well as the activity of key androgenic enzyme 17 $\beta$ -hydrosteroid dehydrogenase. This is in agreement with the reports of (Sadik, 2008) who opined that serum testosterone, luteinizing hormone, follicle stimulating hormone, 17 $\beta$ -HSD and antioxidant profile in testes of the animals were significantly decreased as a result of oxidative stress.

Following various doses administered, cadmium notably altered hormonal and enzymatic status, to the extent that it challenged normal testicular function in rats by inducing



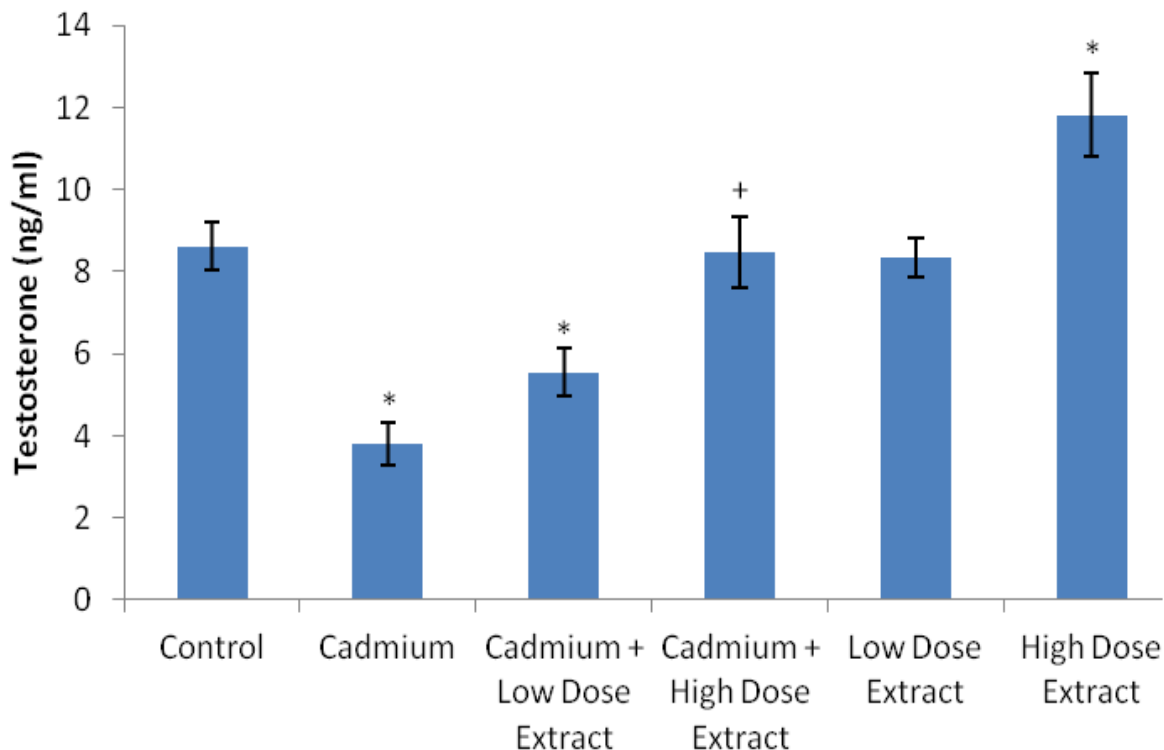
**Figure 2.** Methanol root extract of *C. lutea* on LH in cadmium-induced testicular toxicity in male Wistar rats. \*+ shows significant difference of  $p < 0.05$  when compared with control group and cadmium groups respectively. Low dose extract = 100 mg/kg body weight; High dose extract = 200 mg/kg body weight.



**Figure 3.** Methanol root extract of *C. lutea* on FSH in cadmium-induced testicular toxicity in male Wistar rats. \*+ shows significant difference of  $p < 0.05$  when compared with control group and cadmium groups respectively. Low dose extract = 100 mg/kg body weight; High dose extract = 200 mg/kg body weight.

oxidative stress, damaging testes and altering gonadotropin secretion (Waisberg et al., 2003;

Martynowicz et al., 2005). This is in concord with the report of Waisberg et al. (2003) who maintained that via



**Figure 4.** Methanol root extract of *C. lutea* on testosterone in cadmium- induced testicular toxicity in male Wistar rats. \*+ shows significant difference of  $p < 0.05$  when compared with control group and cadmium groups respectively. Low dose extract = 100 mg/kg body weight; High dose extract = 200 mg/kg body weight.

elevated free radical generation, cadmium could cause testicular distortion and impair male sexual function. It is also in agreement with the reports of Neeven et al. (2007); Bench et al. (1999) and Barbara et al. (2008), asserting that cadmium toxicity would alter sexual function, especially by its harmful action on normal male sexuality. Waalkes et al. (1997); and Lafuente et al. (2001) also reported that the decrease in the testicular function in cadmium toxicity could be added to its effect at the hypothalamic-pituitary-gonadal axis, suggesting that cadmium affects the function of the axis by acting at these three levels.

Testosterone is a vital biomarker of androgenicity (Walton et al., 1995). It is a major androgen secreted by the testes in nature. Approximately 8 mg of testosterone is generated daily; the major source (95%) is known as the interstitial cells of Leydig (Howell and Shalet, 2001). Testosterone is modified to give DHT by 5 $\alpha$ -reductase (Howell and Shalet, 2001). The increase in serum testosterone concentration by methanol extract of *C. lutea* root at 100 and 200 mg/kg body weight is an indication that the extract has androgenic activity by presumably expediting some committed enzymatic step reactions in the testosterone generative pathway. The rescind in levels of testosterone values by the extract of *C. lutea* root suggests that the plant may be indicative of the presence of some androgenic substances which

might have enhanced the productive architecture of testes of the animals to yield more testosterone (Walton et al., 1995).

Luteinizing hormone (LH) is released from cells in the anterior pituitary called gonadotrophs (Yamada et al., 1994) where it binds to Leydig cells in testes to encourage release of testosterone (Stoleru et al., 1993). The increase in serum LH levels by the extract at 100 and 200 mg/kg body weight may mean a stimulatory action by the extract on hypothalamic-pituitary axis of the male rats. Such elevation in LH level may account for the increased level of testosterone since the gonadotropin is responsible for the synthesis and secretion of testosterone (Pitteloud et al., 2008; Valdes-Socin et al., 2017).

Follicle stimulating hormone (FSH) is essential for gonadal enhancement and maturation at puberty as well as gamete synthesis during meiosis (Simoni and Nieschlag, 1995). FSH has a stimulating effect on the testicles where it is essential for normal spermatogenesis. It is also required for the onset and regulation of spermatogenic process. The significant increase in serum concentration of FSH by the extract at 100 and 200 mg/kg body weight may be attributed to stimulatory effect by the extract on the hypothalamic-pituitary axis or anterior pituitary since the release of FSH into the blood is regulated by the gonadotropic releasing hormone

(GnRH) secreted by the hypothalamus (Simoni et al., 1999; Sharma et al., 2012).

17 Beta-Hydroxysteroid dehydrogenase (17 $\beta$ -HSD) catalyzes the enzymatic conversion between highly-active 17 beta-hydroxy- and low-active 17-keto-steroids where it regulates the biological activity of sex hormones (Miettinen et al., 1996). 17 $\beta$ -HSDs catalyzes the reduction of 17-ketosteroids and the dehydrogenation of 17 $\beta$ -hydroxysteroids in steroidogenesis. The major reactions catalyzed by 17 $\beta$ -HSD (e.g., the conversion of androstenedione to testosterone) are hydrogenation (reduction) rather than dehydrogenation (oxidation) reactions (Martel et al., 1992). The increase in activity of 17 $\beta$ -HSD in the testes of the animals by the extract at 100 and 200 mg/kg body weight may be an indication of androgenicity and would boost sexual drive, fertility and reproductive activities in male rats (Labrie et al., 1997).

## Conclusion

The study shows that cadmium-induced oxidative stress altered testicular and gonadotropin secretion by decreasing androgenic hormones and steroidogenic enzyme activities. *C. lutea* however ameliorated the altered testicular indices. The study suggests *C. lutea* as a candidate in the management of penile erectile malfunction and impaired libido especially at 100 and 200 mg/kg body weight.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## **Evaluation of the cytoprotective effects of anti-ulcer agents in acid-alcohol induced gastric ulceration in wistar rats**

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**Adequate studies have been done using proton pump inhibitors and H<sub>2</sub>-receptor antagonist and only few studies for cyto-protective and gastric acid secretions have been done in Nigeria. Therefore this work studied the cyto-protective and gastric acid secretory effects of rabeprazole, ranitidine, omeprazole and cimetidine in wistar rats. 28 male wistar rats of weights 300 to 400 g were recruited and randomly divided into seven experimental groups of 4 rats each. Ulcers were induced via oral administration of a mixture acid alcohol (Ethanol and HCl). Group A: Ulcer alone; Group B: 20 mg/kg Rabeprazole + Ulcer; Group C: 20 mg/kg Rabeprazole + 20 mg/kg Ranitidine + Ulcer. Group D: Normal control group received clean drinking water ad libitum. Group E: 20 mg/kg Omeprazole + Ulcer. Group F: 20 mg/kg ranitidine + ulcer. Group G: 100 mg/kg cimetidine + ulcer. At the end of the treatment and induction, volume of gastric acid secreted, pH values, Ulcer index, stomach and body weights were analyzed statistically. There were significant decrease ( $P<0.05$ ) in the volume of gastric acid secreted for the groups that received the ranitidine and rabeprazole compared to group A (ulcer alone). The pH values of the groups that received the proton pump inhibitors were neutralized at the end of the experiment which shows a better cyto-protective effects of the drugs and there were significant differences ( $P<0.05$ ) among those groups E, F and G compared to group A. The animals with lesser stomach weights have more ulcers index compared to those with higher stomach weights. This research showed that groups treated with a combination of rabeprazole and ranitidine has a better potency for the management of gastric ulcer patients.**

**Key words:** Ulcer, acid-alcohol, Rabeprazole, Ranitidine, Omeprazole, Ranitidine, Wistar rats.

### **INTRODUCTION**

Gastric ulcer is a deep defect in the gastric (stomach) wall penetrating the entire mucosal thickness and muscaris mucosa (Adeniyi et al., 2016). It is the most common prevalent gastrointestinal disorder ever known

accounting 15 mortalities from 15,000 complications yearly in the world. An ulcer in the gastrointestinal tract is a deep necrotic region penetrating the entire mucosal thickness and muscularis mucosae.

Ulcer healing is an active process of filling the mucosal defect with proliferating and migrating epithelial and tissue cells. At the margin, epithelial cells proliferate and migrate onto the granulation tissue to cover (reepithelialize) the ulcer and also invade granulation tissue to reconstruct the glandular structures within the ulcer scar. The epithelialization and reconstruction of glandular structure is controlled by growth factor. Gastric protection means protection against mucosal injury by mechanisms other than inhibition or neutralization of gastric acid (Souza and Dhume, 1991). Protection against mucosal injury includes tight intercellular junctions, mucus secretion and mucosal blood flow, cellular restitution, prostaglandin E<sub>2</sub> epithelial renewal. Drugs such as sucralfate, colloidal bismuth and aluminum containing antacids (Yuan et al., 2015). Gastric ulcers have long been rated as one of the most common diseases affecting humans and young people in particular (Saad et al., 2016).

Peptic Ulcer disease cause high rate of morbidity particularly in the population of non-industrialized countries like Nigeria, where *Helicobacter pylori* affects about 50% of the population (Sidahmed et al., 2013). About 81.4% of the ulcer patients diagnosed with peptic ulcer disease had *H. pylori* infection in south western Nigeria (Adeniyi et al., 2012). Nigeria is listed as an area of high peptic ulcer disease (PUD) prevalence with perforation being most frequent indication for surgery (Felix et al., 2013). Mortality risk for post perforation surgeries in a Nigeria study was found to be 5 to 15% (Sidahmed et al., 2013). PUD is high in sub-Saharan African due to the *H. pylori* and HIV infections that affect about 50% and 15% of the population, respectively (Hestvik et al., 2011). Patients with gastric ulcers are also at risk of developing gastric malignancy (Hansson et al., 1996). Peptic ulcer disease is a looming health challenge in sub-Saharan Africa with over 50% of the population exposed to aggressive factors; hence the need for this research (Saad et al., 2016). PUD is the most common gastrointestinal disease affecting humans with cases of complex surgeries following perforations involving 10% in every 30 hospitalized cases of PUD (Modirat et al., 2018).

Some aggressive factors that causes ulceration include chronic intake of Non-steroidal anti-inflammatory drugs such as aspirin etc, *helicobacter pylori* infection, excessive consumption of alcohol, bile salts, acid and pepsin, severe physiologic stress and some lifestyle factors, tobacco use, stress, depression, anemia, social deprivation, hypersecretory states and genetic factors (Drini, 2017; Parveen and Michael, 2012). This study is aimed at assessing the cytoprotective and gastric acid

secretory effects of rabeprazole, ranitidine, omeprazole and cimetidine in acid-alcohol induced ulcer in wistar rats with the objectives to determining the possible gastric acid protective effect of proton pump inhibitors and H<sub>2</sub>-receptor blockers on acid alcohol induced gastric ulcers in wistar rats.

## MATERIALS AND METHODS

### Drugs and chemicals

Drugs used for this research work were purchased from Open heaven pharmacy limited. Parklane avenue, G.R.A, Enugu, while the chemicals of analytical grades were obtained from Sigma Aldrich USA.

### Experimental animals

A total of 28 male adult Wistar rats of weights between 150 to 300 g were purchased from the animal house unit of the College of Medicine, Enugu State University of Science and Technology, Parklane Enugu. The research was conducted in the Research Laboratory Animal House Unit of the College of Medicine, Enugu State University of Science and Technology, Parklane Enugu. The rats were housed in standard rat's cages and acclimatized for 7 days with a 12 h dark/light cycle at a temperature of 26.1±2°C and relative humidity of 56±0.2 during which they were provided with hybrid feed and clean tap drinking water *ad libitum*.

### Experimental design

The 28 wistar rats were divided into seven experimental groups of four rats each and they include;

Group 1: Ulcer alone; Group 2: 20 mg/kg Rabeprazole + Ulcer; Group 3: 20 mg/kg Rabeprazole + 20 mg/kg Ranitidine + Ulcer; Group 4: Normal control group received clean drinking water *ad libitum*; Group 5: 20 mg/kg Omeprazole + Ulcer; Group 6: 20 mg/kg ranitidine + Ulcer and Group 7: 100 mg/kg Cimetidine +Ulcer.

### Methods used in the induction of ulcers

The animals were anesthetized with intraperitoneal (IP) administration of 25% Urethane (Sigma Aldrich USA) at a dose of 0.6 ml/100 g one hour before induction of ulcer. The induction of ulcer was done according to the method of [7]. Ulcer was induced by oral administration of 1 ml/200 g of acid alcohol (50% solution of absolute ethanol and 50% solution of HCl) through oesophago-gastric cannula.

### Measurement of gastric acid secretion and gastric pH

After anesthesia, the trachea was cannulated using polyethylene oral tubing after an incision was made at the trachea. The oral tube inserted into the trachea and then ligated using a thread to ensure

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proper aeration of the animal during the experimental period (Adeniyi et al., 2012). Abdomen was opened and the gastric content evacuated, followed by ligation of the pylorus.

The animals were given 1 hr to acclimatize before collection of basal acids secretion. The basal acid secretions were collected 10 ml/15 mins, from each animal and recorded accordingly. 4 hrs after acid-alcohol administration, stomach contents were collected and centrifuged at 1000 rpm for 15 mins. The volume of the supernatants were measured and expressed as ml/100 g and the pH of the supernatants was measured using a digital pH meter according to the method of (Modirat et al., 2018).

#### Determination of gastric acid output

The gastric acid output was determined in the supernatant (2 ml) by titration with 0.0025N NaOH using Toepfers reagent as indicator. The concentration of the acid were calculated using the formula below according to the method of (Saheed et al., 2015).

$$M_a V_a = M_b V_b$$

$$M_a = \frac{M_b V_b}{V_a}$$

where,  $M_a$  = Normality of acid in effluent sample,  $V_a$  = Volume of acid in effluent sample,  $M_b$  = Normality of base (NaOH = 0.0025N) and  $V_b$  = Volume of base (NaOH).

#### Cyto-protective studies

Briefly cleaned stomachs were pinned on a corkboard and the ulcer were scored using dissecting microscope with square grid eyepiece based on grading on 0 to 5 scale (depicting severity of vascular congestion lesions/hemorrhage as presented. 0 means no lesion. 1 represents vascular congestion, 2, 3, 4 and 5 represents one or two lesions, severe lesions, very severe lesions and mucosa full of lesions of marked sizes, respectively (Saheed et al., 2015).

#### Mean ulcer scores/indices

Mean ulcer scores for each animal were expressed as ulcer index (U.I) and the percentage of inhibition against ulceration was determined using the expression by (Szabo and Hollander, 1995).

$$U.I = \frac{\text{ulcerated area} \times 100}{\text{total stomach area} \quad 1}$$

#### Statistical analysis

Data were presented as mean  $\pm$  standard error of mean (SEM). Data were analyzed using statistical computer software (SPSS version 21) one way analysis of variance (ANOVA) with Tukey post hoc test for further multiple comparisons. Value of  $p < 0.05$  was considered to be statistically significant except otherwise stated.

#### Ethical clearance

The experiment was approved by the Animal Research Ethics

Committee of the College of Medicine, Enugu State University of Science and Technology, Enugu and handling of animals followed the internationally accepted procedures according to the Institute of Laboratory Animal Research guide for the care and use of laboratory Animals.

## RESULTS

Table 1 shows the result of the basal volume of gastric acid secreted at the 10 mins intervals for 30 mins. At the 10th min of gastric acid secretion, it can be observed from the table that group A secreted the maximal volume of gastric acid while the least volume was secreted by group E. There were significant differences ( $P < 0.05$ ) among all the groups compared with group A (Ulcer alone), at the end of the 10th min of secretion.

The effluent was in a decreasing order starting from group A followed by group B and C respectively but on the 20 mins, group A also recorded the highest volume (1.00 $\pm$ 0.42) ml with group D as the least. Hence, at the 30th min, group A (1.550 $\pm$ 0.71 ml) still recorded the highest volume of gastric acid secreted followed by group B as usual. There was no significant difference ( $P > 0.05$ ) at the 20th and 30th mins of the gastric acid secretion respectively. Table 2 shows the volume of the gastric acid secreted after one hour post treatment and induction of the gastric ulcer by the use of acid alcohol.

After the first 10 mins of the post ulcer induction, the volume of gastric acid secreted was more in group F (0.9 $\pm$ 0.2 ml), followed in a decreasing order by group B (0.78 $\pm$ 0.29 ml), G (0.74 $\pm$ 0.46 ml), E (0.68 $\pm$ 0.38) etc., as seen in the table 2 below. Hence, at the end of the 20th mins of post treatment and induction of ulcer, it was observed that group A (0.90 $\pm$ 0.96 ml), recorded the highest volume with the least value in group B and E with values of 0.40 $\pm$ 0.18 and 0.40 $\pm$ 0.20 ml respectively. At the 30th mins, the volume secreted was more in group A (ulcer alone) group without anti-ulcer agents with the least recorded by group by group E (0.35  $\pm$ 0.10 ml). There was no significant ( $P > 0.05$ ) difference among the groups.

Table 3 also shows the basal pH levels of gastric acid secreted by all the experimental groups at different time intervals. At the 10th min, the pH of the solution still falls within the normal physiological ranges for gastric acid. Groups A, B and C were more acidic than groups E, F and G at the 10th min. The same patterns were observed in the 20th and 30th mins, respectively. There were significant differences ( $P < 0.05$ ) among some of the groups compared with one another. At the 20th min of basal secretion, there were significant differences ( $P < 0.05$ ) among groups E and G compared with group C but on the 30th min, there was significant difference between group E and F compared with group C respectively. Moreover, Table 4 shows the result of the pH after post induction of ulcer and treatment of administration of the drugs. Group A was found to be

**Table 1.** Result of the mean  $\pm$  sem basal gastric acid secretion at different time intervals.

Group	10th min	20th min	30th min
A	2.85 $\pm$ 1.63	1.00 $\pm$ 0.42	1.50 $\pm$ 0.71
B	0.75 $\pm$ 0.42 <sup>*</sup>	0.48 $\pm$ 0.31	0.58 $\pm$ 0.55
C	0.70 $\pm$ 0.45 <sup>*</sup>	0.80 $\pm$ 0.48	1.00 $\pm$ 1.01
D	0.63 $\pm$ 0.43 <sup>*</sup>	0.30 $\pm$ 0.14	0.40 $\pm$ 0.14
E	0.30 $\pm$ 0.00 <sup>*</sup>	0.45 $\pm$ 0.24	0.40 $\pm$ 0.16
F	0.38 $\pm$ 0.05 <sup>*</sup>	0.50 $\pm$ 0.40	0.40 $\pm$ 0.14
G	0.48 $\pm$ 0.19 <sup>*</sup>	0.40 $\pm$ 0.18	0.40 $\pm$ 0.22

Results were expressed as Mean $\pm$  Standard Error of Mean; (n=4), Values with superscripts showed a statistical significant difference; <sup>\*</sup>P<0.05 compared with A, <sup>†</sup>P<0.05 Compared with B, <sup>‡</sup>P<0.05 Compared with C, <sup>§</sup>P<0.05 Compared with D, <sup>¶</sup>P<0.05 Compared with E, <sup>®</sup>P<0.05 Compared with group E . <sup>ˆ</sup>P<0.05 Compared with F and <sup>ˉ</sup>P<0.05 Compared with G, respectively.

**Table 2.** Result of the mean  $\pm$  sd volume of gastric acid secretion at different time intervals after drug administration and induction of ulcer.

Group	10th min	20th min	30th min
A	0.57 $\pm$ 0.47	0.90 $\pm$ 0.96	1.47 $\pm$ 0.19
B	0.78 $\pm$ 0.29	0.40 $\pm$ 0.18	0.90 $\pm$ 0.38
C	0.48 $\pm$ 0.25	0.48 $\pm$ 0.31	0.45 $\pm$ 0.31
D	0.45 $\pm$ 0.19	0.45 $\pm$ 0.24	0.40 $\pm$ 0.16
E	0.68 $\pm$ 0.38	0.40 $\pm$ 0.20	0.35 $\pm$ 0.10
F	0.90 $\pm$ 0.26	0.38 $\pm$ 0.05	0.65 $\pm$ 0.44
G	0.74 $\pm$ 0.46	0.43 $\pm$ 0.09	0.40 $\pm$ 0.22

Results were expressed as Mean $\pm$  Standard deviation; (n=4); Values with superscripts showed a statistical significant difference; <sup>\*</sup>P<0.05 compared with A; <sup>†</sup>P<0.05 Compared with B; <sup>‡</sup>P<0.05 Compared with C; <sup>§</sup>P<0.05 Compared with D; <sup>¶</sup>P<0.05 Compared with E; <sup>®</sup>P<0.05 Compared with group E . <sup>ˆ</sup>P<0.05 Compared with F and <sup>ˉ</sup>P<0.05 Compared with G, respectively.

more acidic followed by group D and B respectively. Groups E and F were slightly alkaline and group G showed a neutral pH of 7.0. There were significant differences (P<0.05) among all the groups compared with group A as shown in the table 4 below. Hence, at the end of the 30th mins, Group A still maintained its acidic nature with a PH of 4.83 $\pm$ 0.29 while group C became totally alkalinized with a pH of 8.10  $\pm$ 0.00 as seen in the table below. Group E, F and G were neutralized with PH stabilized at 7.00.

Table 5 shows the rats in group D have the highest body weights (405 $\pm$ 29.06 g) and this was followed by rats in groups C, A, B,G and E with group F as the least weight. The body weights were compared statistically and there was no significant difference (P>0.05) among the groups. The stomach weights did not follow similar pattern as the body weight as the stomach weight recorded the highest weight in group B and C

respectively. They were followed by groups A, G, D and E with group E as the least in their stomach weights values. There was also a significant difference (P<0.05) between group E when compared with group B. From the table, it has been reported that animals with smaller stomach weight has more ulcers than those with higher stomach weight and there is a correlation as depicted by the ulcer index table and that of the stomach weight.

From Table 6, it was observed that 3 rats in group A have multiple linear ulcer of mark size and one hemorrhagic erosion greater than 5 mm size while in group B, one rats has hemorrhagic erosion of less than 5 mm and no ulcer was observed in group C and D. In group E, two rats have hemorrhagic erosion of less than 5mm and one rat has hemorrhagic erosion greater than 5 mm and no ulcer was seen in rats 4 as presented in the table above. In group F, three rats have hemorrhagic erosion of less than 5 mm while one rat has hemorrhagic

**Table 3.** Result of the mean  $\pm$  standard error of mean of the basal pH values of gastric acid secretion at different time intervals before drug administration and induction of ulcer.

Group	10th Min	20th Min	30th Min
A	6.00 $\pm$ 0.50	6.50 $\pm$ 0.50	6.33 $\pm$ 0.29
B	6.13 $\pm$ 0.25	6.13 $\pm$ 0.25	6.25 $\pm$ 0.29
C	5.13 $\pm$ 0.25 <sup><math>\beta</math></sup>	5.75 $\pm$ 0.50	5.63 $\pm$ 0.25
D	6.88 $\pm$ 0.25 <sup><math>\gamma</math></sup>	6.75 $\pm$ 0.50	6.63 $\pm$ 0.75
E	7.50 $\pm$ 0.58 <sup><math>\alpha\beta\gamma</math></sup>	7.00 $\pm$ 0.82 <sup><math>\gamma</math></sup>	7.25 $\pm$ 1.26 <sup><math>\gamma</math></sup>
F	7.50 $\pm$ 0.58 <sup><math>\alpha\beta\gamma</math></sup>	7.00 $\pm$ 0.00 <sup><math>\gamma</math></sup>	7.25 $\pm$ 0.50 <sup><math>\gamma</math></sup>
G	7.00 $\pm$ 0.00 <sup><math>\gamma</math></sup>	7.25 $\pm$ 0.50 <sup><math>\gamma</math></sup>	7.00 $\pm$ 0.00

Results were expressed as Mean $\pm$  Standard Error of Mean; (n=4), Values with superscripts showed a statistical significant difference; \*P<0.05 compared with A,  <sup>$\beta$</sup> P<0.05 Compared with B,  <sup>$\gamma$</sup> P<0.05 Compared with C,  <sup>$\delta$</sup> P<0.05 Compared with D,  <sup>$\epsilon$</sup> P<0.05 Compared with E,  <sup>$\zeta$</sup> P<0.05 Compared with group E.  <sup>$\eta$</sup> P<0.05 Compared with F and  <sup>$\theta$</sup> P<0.05 Compared with G, respectively.

**Table 4:** Result of the mean  $\pm$  standard error of mean of the pH values of gastric acid secretion after one hour post induction of ulcer.

Group	10th Min	20th Min	30th Min
A	4.50 $\pm$ 0.50	4.83 $\pm$ 0.29	4.83 $\pm$ 0.29
B	6.38 $\pm$ 0.25	6.25 $\pm$ 0.29*	6.25 $\pm$ 0.29*
C	8.13 $\pm$ 0.25 <sup><math>\beta</math></sup>	7.25 $\pm$ 0.96*	8.00 $\pm$ 0.00*
D	5.88 $\pm$ 0.25 <sup><math>\gamma</math></sup>	5.88 $\pm$ 0.25 <sup><math>\gamma</math></sup>	6.00 $\pm$ 0.00*
E	7.75 $\pm$ 0.50 <sup><math>\alpha\beta\delta</math></sup>	7.50 $\pm$ 0.58 <sup><math>\alpha\beta\gamma</math></sup>	7.75 $\pm$ 0.96 <sup><math>\gamma</math></sup>
F	7.25 $\pm$ 0.50 <sup><math>\alpha\beta\delta</math></sup>	7.25 $\pm$ 0.50 <sup><math>\alpha\delta</math></sup>	7.00 $\pm$ 0.00 <sup><math>\alpha\delta</math></sup>
G	7.00 $\pm$ 0.00 <sup><math>\alpha\delta</math></sup>	7.00 $\pm$ 0.00 <sup><math>\alpha</math></sup>	7.00 $\pm$ 0.00 <sup><math>\alpha\delta</math></sup>

Results were expressed as Mean $\pm$  Standard Error of Mean; (n=4), Values with superscripts showed a statistical significant difference; \*P<0.05 compared with A,  <sup>$\beta$</sup> P<0.05 Compared with B,  <sup>$\gamma$</sup> P<0.05 Compared with C,  <sup>$\delta$</sup> P<0.05 Compared with D,  <sup>$\epsilon$</sup> P<0.05 Compared with E,  <sup>$\zeta$</sup> P<0.05 Compared with group E.  <sup>$\eta$</sup> P<0.05 Compared with F and  <sup>$\theta$</sup> P<0.05 Compared with G, respectively.

**Table 5.** Result of the mean  $\pm$  standard error of mean of the stomach and body weights after drug administration and induction of ulcer.

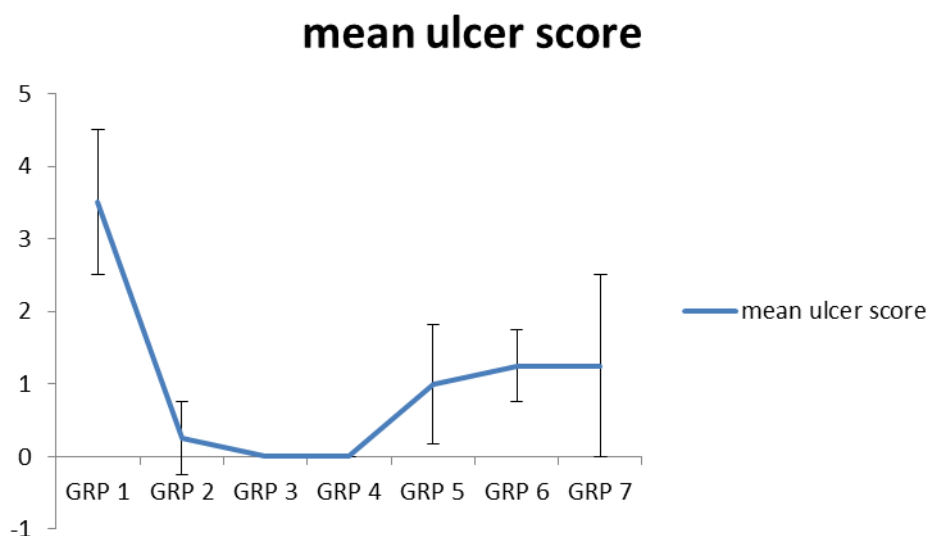
Group	Stomach weight (gram)	Body weights (gram)
A	2.78 $\pm$ 0.21	378.50 $\pm$ 49.9
B	3.42 $\pm$ 0.32	351.75 $\pm$ 10.9
C	3.14 $\pm$ 0.29	388.25 $\pm$ 42.16
D	2.46 $\pm$ 0.57	405.00 $\pm$ 29.06
E	2.11 $\pm$ 0.47 <sup><math>\beta</math></sup>	331.25 $\pm$ 13.28
F	2.28 $\pm$ 0.12	332.38 $\pm$ 19.3
G	2.58 $\pm$ 1.02	348.00 $\pm$ 41.67

Results were expressed as Mean $\pm$  Standard Error of Mean; (n=4), Values with superscripts showed a statistical significant difference; \*P<0.05 compared with A,  <sup>$\beta$</sup> P<0.05 Compared with B,  <sup>$\gamma$</sup> P<0.05 Compared with C,  <sup>$\delta$</sup> P<0.05 Compared with D,  <sup>$\epsilon$</sup> P<0.05 Compared with E,  <sup>$\zeta$</sup> P<0.05 Compared with group E.  <sup>$\eta$</sup> P<0.05 Compared with F and  <sup>$\theta$</sup> P<0.05 Compared with G, respectively.

**Table 6.** Result of the ulcer score/index (mm).

Group	Rat 1	Rat 2	Rat 3	Rat 4	Total score	Ulcer index
A	2	4	4	4	14	3.5±1.00
B	0	1	0	0	1	0.25±0.50**
C	0	0	0	0	0	0.00±0.00**
D	0	0	0	0	0	0.00±0.00**
E	1	1	2	0	4	1.00±0.82**
F	1	1	1	2	5	1.25±0.50**
G	1	3	1	0	5	1.25±1.26**

Key: 0 = No Ulcer; 1= Hemorrhagic erosions < 5mm; 2= hemorrhagic erosion >5mm; 3= Many small linear ulcers > 2mm ); 4= Multiple linear ulcers of mark size. 5= Mucosa full of lesion with marked sizes.



**Figure 1.** Result of the mean ulcer score in wistar rats treated with comparative evaluation of anti-ulcer agents in acid-alcohol induced ulceration in wistar rats. (n=2)

erosion greater than 5 mm. Finally, in group G, rat 2 has many linear ulcers of smaller sizes that are greater than 2 mm while two rats have hemorrhagic erosion of less than 5 mm and no ulcer was seen in rat 4 of the same group.

Figure 1 represents the mean±SD of the ulcer score/index for all the experimental groups. From the graph, the ulcer score was more pronounced in group 1 with mean value of 3.5±1.00 and this was followed by group F and G with values of 1.25± 1.26 and 1.25±0.5, respectively. Groups C and D recorded the least ulcer score. These differences in ulcer indices were compared statistically and observed that there was significant difference (P<0.05) between groups B to G compared to group A (Ulcer alone).

## DISCUSSION

This research work compared the cytoprotective effects

of some antiulcerative agents (rabeprazole, ranitidine, omeprazole and cimetidine, etc.) on acid alcohol induced gastric ulceration in Wistar rats. The objectives involve determining the possible gastric acid protective effect of proton pump inhibitors and H<sub>2</sub>-Receptor blockers and to assess their possible synergistic effects on acid alcohol induced gastric ulcers in wistar rats. In this present study, the significant increase in ulcer index and gastric acid volume following oral administration of cimetidine and ranitidine may be attributed to either free radical formation or inhibition of prostaglandin synthesis (Parveen and Michael, 2012).

Decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric acid secretion which are important event in the etiology of mucosal ulceration (Parveen and Michael, 2012). This agrees with the reports of Saheed et al. (2015) and Szado and Hollander (1995), where indomethacin was reported to have caused alteration in gastric secretion of

rats. Conversely, pretreatment with the antiulcer agents significantly reduced these parameters. In fact, the effect noticed with pH compared favorably with normal control and indeed suggestive of the possible gastroprotective attributes of the anti-ulcer agents. A combination of events including released preformed mucus, wound retraction and epithelization is involved in ulcer protective process after toxicological injury (Modirat et al., 2018; Bech et al., 2000).

Besides, providing significant buffering capacity for neutralization of luminal acids, the mucus also offer protection against both endogenous aggressors and exogenous gastro toxic agents such as acid-alcohol, thereby enhancing the rate of local healing process. In this study, decreased cyto-protective activity in acid alcohol ulcerated rats indicated reduced protective ability of the mucosal membrane against hemorrhagic erosion, thus resulting in tissue damage. This implied the decreased ability of gastric mucosa to withstand the offensive onslaught of acid alcohol. Thus antiulcer drugs such as rabeprazole, ranitidine, used, inhibit the secretion and synthesis of gastric acid and thus protects against gastric ulcer damage and this could be attributed to its synergistic effects.

Pre-treatment with the antiulcer agents however gave cyto-protective effects which is associated with decreased pepsin activity and elevated mucus level in the gastric mucosa. Thus these drugs shielded the gastrointestinal membrane by abrogating the catastrophic influence of acid alcohol in ulcerative rats (Bech et al., 2000). This is indication of enhanced mucus secretory potential of the drugs and suggestive of their significant role in cyto-protection against gastric acid damage to the mucosal endothelium. Cyto-protection of mucosa epithelial cells was prominently displayed by a synergistic administration of 20 mg/kg rabeprazole and ranitidine thereby depicting a better cyto-protective capacity than cimetidine and ranitidine alone.

## Conclusion

The attenuation of gastric affronts of acid alcohol by the administration of 20 mg/kg dose of a combination of Rabeprazole and ranitidine showed an excellent cyto-protective effect on the gastric mucosa of wistar rats. These cyto-protective effects of the combination of Rabeprazole and Ranitidine could be a possible synergistic efficacy.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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