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ARTICLE

Effect of 90 Days Sub-chronic Administration of Omeprazole (A Proton Pump Inhibitor) on Haematological, Biochemical, Antioxidant, Reproductive and Histopathological Parameters in Male and Female Rats

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Abstract
Despite the widespread use of omeprazole (OME), there are still concerns about its long-term safety and tolerability, especially with long-term or indiscriminate use. This study was designed to evaluate the effect of 90 days sub-chronic administration of OME on haematological, biochemical, antioxidant, reproductive and histopathological parameters in male and female rats. Forty-eight (48) Wistar rats of both sexes were divided into 4 groups of 6 male and 6 female animals each. Group I received 10 mL/kg normal saline, while Groups II – IV received 0.12 mg/kg, 0.57 mg/kg, 2.85 mg/kg of OME respectively for 90 days. At the end of 90 days, 5 mL blood sample was collected, and vital organs were harvested for relevant analyses. In this study, OME did not elicit any significant ($P>0.05$) change in the body weight or weight of vital organs, but elicited a significant ($P<0.05$) reduction in sperm count at the supratherapeutic dose (2.85 mg/kg). The supratherapeutic dose of OME produced significant increase in cholesterol ($P<0.0001$) and LDL ($P<0.0001$) in female rats. OME did not generally produce a significant change in haematological parameters. OME elicited a significant decrease in CAT ($P<0.05$) in female and combined animals. Additionally, histopathological presentations of vital organs were generally non-abnormal. From this study, it can be concluded that long term administration of OME is generally safe and tolerable, especially at lower doses. Findings from this study suggest that OME may potentially induce changes in sperm characteristics (in relation to male rats), hepatoxicity, hypercholesteremia, hyperlipidemia and thrombocytosis over a long duration, at higher doses and especially in female animals.

Keywords: Omeprazole; Chronic toxicity; Side effects; Gastric ulcer; Proton pump inhibitor; Toxicological assessment

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1.0 Introduction

Omeprazole, a proton pump inhibitor, is not a new drug in medical and pharmaceutical circles. Its use for the management of gastric ulcer and gastroesophageal reflux disease (GERD) since its discovery and launch in the 1980's is well documented (Olbe et al., 2003; Lundell, 2015). Today, omeprazole (OME) has become one of the most frequently prescribed and widely used medications (Forgacs and Loganayagam, 2008; Forgerini et al., 2018). It may also have become a form of standard especially in terms of cost-efficient and clinically effective therapy for gastric ulcer and GERD (Bate et al., 1999; Fass et al., 1999). Furthermore, OME (and other proton pump inhibitors) is considered a mainstay in the control of disease manifestations and improvement of quality of life of patients with GERD (Attwood et al., 2015). This is because it is very effective in suppressing acid secretion (its basic mechanism of action) and has a wide margin of safety (Savarino et al., 2009; Weersink et al., 2018). The safety profile of OME has been well established and documented through several clinical trials and over 30 years of post-market exposure and surveillance (Attwood et al., 2015). Indeed, the rate of occurrence of any adverse event with OME has been described to be few (Arnold and Koop, 1989), to reduce with time and no previously unidentified adverse event occurred with long term OME administration (Joelson et al., 1992). It is therefore plausible to posit that OME is safe and well tolerated in both short- and long-term administration (Arnold and Koop, 1989; Joelson et al., 1992; Savarino et al., 2009; Li et al., 2013). The side effects of OME such as headache, diarrhea, nausea, and rash are reportedly mild, non-life threatening and serious adverse events are rare (Thomson et al., 2010).

However, this submission on the overall safety of OME, which has been the premise for its widespread usage, may not be entirely accurate especially in long-term use. Firstly, lifelong toxicological studies conducted with high doses of OME in rats led to the development of carcinoids (endocrine tumours) in the stomach of test rats and this led to the halting of all OME clinical trials in 1984, before OME was released and launched for use (Olbe et al., 2003). The clinical studies were restarted after a possible explanation of the role of gastrin (whose secretion increased as the amount of gastric acid secretion was decreased by OME) in the development of the carcinoids was elucidated (Olbe et al., 2003). Regardless, long term use of OME is associated with induction of DNA damage (Neumann, 2009; Downes and Foster, 2015) and increased risk of gastric cancer (Abbas et al., 2019).

Secondly, long-term use of OME (and other PPIs) has been associated with increased possibility of respiratory infections (like pneumonia), enteric infections (like *Clostridium difficile*) and nutritional deficiencies which may lead to bone fractures and hypomagnesaemia (Laheij et al., 2004; Ali et al., 2009; Hess et al., 2012; Moayyedi and Leontiadis, 2012; Reimer, 2013; Zhou et al., 2016). More recently, concerns about the safety of OME and the serious adverse effects it may cause have been raised (Weersink et al., 2018; Abbas et al., 2019), leading to several warnings issued by the United States Food and Drug Administration on the use of OME and PPIs in general (United States Food and Drug Administration, 2017).

With its extended duration of action in decreasing gastric acid secretions, OME has been designed to be taken as a single daily dose, sometimes for up to 8 weeks depending on the situation or indication (United States Food and Drug Administration, 2012). However, indiscriminate and extended use of OME beyond 8 weeks is common (Abbas et al., 2019). This problem is predicated on the relative ease of over-the-counter accessibility and availability of low cost generic OME to patients leading to inappropriate usage (Abbas et al., 2019). This problem becomes bigger in low- and middle-income countries with broken health care system where prescriptions are not well enforced, and pharmacovigilance efforts maybe sub-optimal (Kigubaet al., 2023).
Inappropriate usage of OME is further compounded by indiscriminate prescription by physicians who routinely prescribe OME to patients without any validated indication (Heidelbaugh et al., 2012) and continuation of OME therapy by primary care physicians without proper assessment (Scarpignato et al., 2016). Mastroianni et al. (2009) found that OME is one of the most commonly used drugs associated with hospital admission due to adverse drug reactions. Additionally, OME has been associated with frequent and long-term off label use (Chen et al., 2014). All these lead to unwarranted and prolonged use of OME which may potentially expose patients to adverse events.
As a result of all of this, it is important that the long-term safety and tolerability of OME be appraised and confirmed again as a call to action to prevent potential adverse events associated with its long-term use and based on the warnings issued by the United States FDA on the use of OME (and other proton pump inhibitors). Therefore, this study was designed to contribute to medical and academic knowledge on the safety or potential dangers of long-term administration of OME, as well as the variation of effects between male and female sexes.
This study evaluated the effect of 90 days sub-chronic administration of OME on haematological, biochemical, antioxidant, reproductive and histopathological parameters in male and female rats.

2.0 Experimental

Drugs and Experimental animals: Generic omeprazole capsules (Meprasil, Fidson Nigeria Limited, Lagos, Nigeria) and normal saline fluid (Juhel Nigeria Ltd., Lagos, Nigeria) were procured from a local Pharmacy in Lagos, Nigeria. Forty-eight (48) Wistar rats of both sexes and weighing between 150 - 200 g were procured from the Laboratory Animal Centre of the College of Medicine, University of Lagos (CMUL), Lagos, Nigeria. The test animals were housed in plastic cages under standard environmental conditions of 12-hour light/12-hour dark cycle and standard temperature of 23 - 28^{o}C. The test animals were allowed to adapt to the new environment for two weeks before experimentation began. They were provided with drinking water and standard rodent diet (Livestock Feeds Plc., Lagos, Nigeria) as needed. Animal treatment and experimental protocol for this study were in accordance with the provisions of the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 2011) and an approved protocol (CMUL/HREC/08/18/415).

Experimental Design and Treatment: Test animals were divided into 4 groups of 6 male and 6 female animals each. Male and female animals within the same group were housed in separate cages. Different working concentrations of OME representing 0.114 mg/kg, 0.57 mg/kg, and 2.85 mg/kg were prepared by dissolving OME in normal saline. These working concentrations were based on extrapolation from human daily dose of 40 mg OME (United States Food and Drug Administration, 2012) and represents one-fifth of therapeutic dose (sub-therapeutic dose), therapeutic dose and five-times therapeutic dose (supratherapeutic dose) of OME. This study followed established protocol for toxicological studies (Yemitan and Adeyemi, 2004; Amida et al., 2007; Afolabi et al., 2012).
Group I received 10 mL/kg of normal saline, while Groups II – IV received 0.114 mg/kg, 0.57 mg/kg, and 2.85 mg/kg of OME respectively for 90 days. Feed and water intakes were measured weekly and test animals were weighed weekly during the treatment period. Additionally, test animals were generally observed for behavioural changes and mortality during the treatment period.

Blood and Organ Collection: On day 90, test animals were fasted while drinking water was made available as needed. On day 91, the weight of the test animals was measured and 6 test animals from each group (3 males and 3 females) were randomly selected and anaesthetized. Under anaesthesia, 5 mL of blood sample was collected through the retro-orbital plexus vein of each rat. 2.5 mL of whole blood was collected in plain bottles, centrifuged, serum extracted and used for biochemical and antioxidant tests. Another 2.5 mL of blood was collected in EDTA-treated bottles for haematological tests. After this, the animals were terminated through cervical dislocation, dissected

and vital organs (liver, heart, lungs, spleen, kidneys, brain, testes, and pancreas) were harvested. The vital organs were weighed, and representative samples were fixed in 10% formol-saline for histopathological assessment. Cut tissue sections of kidneys, ovary, testes, liver, and brain were rinsed in potassium chloride and used for in-vivo antioxidants and MDA determinations.

Food and Water Intakes: Food and water intakes by test animals were measured weekly to determine the effect of sub-chronic administration of OME on food and water intakes by test animals.

Body and Organ Weight Change in Test Animals: Body weight of test animals was measured weekly to determine the effect of sub-chronic administration of OME on body weight of test animals. The % weight change was calculated as [(Difference between interval and initial body weight)/initial body weight] ×100. The weight of harvested vital organs was measured after termination of test animals to evaluate the effect of sub-chronic administration of OME on changes in the weight of vital organs.

Sperm Analysis: Seminal fluid was collected from male rats for the estimation of sperm motility, count and morphology in accordance with the methods of Cheesbrough (2005) and Ogli et al. (2009). Sperm motility was accessed by placing 10 µL of harvested semen on a slide covered with glass. This was observed under Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany) for the number of motile cells per total of 100 spermatozoa.
Estimation of sperm count was carried out by using a 1 in 20 dilution of semen with sodium bicarbonate-formalin and filled in an improved Neubauer ruled chamber using a Pasteur pipette. Using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany), the number of spermatozoa was counted after 3-5 min. The number of spermatozoa in 1 mL of seminal fluid was then estimated by multiplying the number counted with 100 000.
Finally, sperm morphology was accessed using a thin smear of diluted semen on a slide fixed with 95% v/v ethanol, air dried, and washed with sodium bicarbonate-formalin solution. This was rinsed with water, covered with dilute carbon fuchsin (1 in 20) followed by Dilute Loeffler's methylene blue (1 in 20), and allowed to dry. Normal and abnormal spermatozoa were examined using Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany) and the percentage of normal and abnormal morphology was estimated.

Biochemical Assessment: Blood samples collected were analysed for levels of aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphate (ALP), total bilirubin (TB), conjugated bilirubin (CB), total protein (TP), albumin (ALB), cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), uric acid, urea and creatinine using Roche and Cobas commercial kits and Roche/Hitachi 904 automated analyzer (Roche Professional Diagnostics, Basel, Switzerland). The electrolyte (potassium, chloride and sodium) levels were also analyzed.

Haematological Assessment: The levels of leucocytes (WBC), lymphocyte differentials (LYM%), differential from other white blood cells (MID%), granulocyte differentials (GRAN%), haemoglobin (HGB), erythrocyte (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) were determined using automated haematology analyzer (Sysmex Haematology-Coagulation Systems, Model KX-21N, Sysmex Incorporation, Kobe, Japan).

Estimation of Serum and Tissue In-vivo Antioxidants and MDA Levels: Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels in the blood and harvested tissues (kidneys, ovary, testes, liver, and brain) were estimated using established protocols as reported by Soon and Tan (2002).

Histopathological Assessment: Harvested vital tissues were fixed in 10% formol-saline, dehydrated in graded alcohol, embedded in paraffin and cut into 4-5 µm sections. The cut sections were then stained with haematoxylin-eosin for photomicroscopic assessment using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany) and protocol as reported by Ishola et al. (2012).
Data and Statistical Analysis: All results from this study are presented as mean ± S.E.M. All results were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was set at P-value < 0.05.

3.0 Results

Effect of OME on Food and Water Intakes: At the dose of 2.85 mg/kg, OME elicited a significant increase (P<0.001) in food intake in both male and female test animals. OME at all doses did not elicit any significant effect on water intake (Table 1).

Effect of OME on Body Weight and Vital Organs Weight Change: OME at all doses did not elicit any significant (P>0.05) change in the body weight of test animals (Figure 1). OME did not generally elicit any significant change in the weight of heart, spleen, kidneys, brain, testes, ovary and pancreas. However, 0.57 mg/kg OME produced a significant (P<0.05) elevation in the weight of the liver in female test animals (Figure 2) and the lungs in both male and female test animals at 0.114 mg/kg (Figure 3).

Effect of OME on Sperm Motility, Count and Morphology: OME at the supratherapeutic dose of 2.85 mg/kg produced a significant reduction (P<0.05) in sperm count in male animals (Figure 4), while it produced no significant effect on sperm motility and morphology (% abnormality).

Effect of OME on Biochemical Parameters and Electrolyte Levels: OME produced a significant increase in the levels of ALP (P<0.01, 0.114 mg/kg OME) and ALT (P<0.05, 2.85 mg/kg OME) in female test animals. OME at the supratherapeutic dose of 2.85 mg/kg elicited a significant increase (P<0.0001) in the levels of cholesterol and LDL in female animals. Additionally, OME at the supratherapeutic dose elicited a significant increase in cholesterol (P<0.01) and LDL (P<0.001) levels in combined (male + female) test animals (Tables 2 and 3). OME did not elicit any significant effect on electrolyte levels in male and female rats (Table 3).

Effect of OME on Haematological Parameters: OME did not generally produce a significant change in haematological parameters, except for a significant increase (P<0.01) in platelet levels in female animals at the therapeutic and supratherapeutic doses (Table 4).

Effect of OME on Antioxidant Indices in the Blood and Vital Organs: OME elicited a significant decrease in blood levels of CAT (P<0.05) in female and combined animals at the sub-therapeutic dose (0.114 mg/kg). Additionally, OME at all doses generally elicited a non-significant decrease in blood antioxidant levels of GSH and SOD in both male and female animals (Table 5). In respect of the brain, OME elicited significant increase in GSH level (P<0.05) at the sub-therapeutic dose, but elicited a decrease in CAT level (P<0.01) at the therapeutic dose in male animals (Figures 5 and 6). Omeprazole at the therapeutic dose significantly (P<0.05) increased SOD level in the kidney of female rats (Figure 7).

Effect of OME on histopathological assessment
In the brain, ovary, testes, pancreas, and spleen tissues, OME at all doses did not produce any histopathological abnormalities in male and female animals. In the heart, myocardial inflammation was observed in female animals at the dose of 2.85 mg/kg OME. No abnormality was observed in male animals. In the kidney, vascular congestion was observed in female animals at the dose of 2.85 mg/kg OME. No abnormality was observed in male animals. In the lungs, moderate pulmonary inflammation was observed at the dose of 0.114 mg/kg in female animals, while moderate to severe pulmonary inflammation was observed across all doses in male animals. In the liver, sinusoidal congestion was observed in both male (2.85 mg/kg OME dose) and female animals (0.57 and 2.85 mg/kg OME doses) (Figures 8-13).

Effect of OME on mortality
No mortality was observed in this study.

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Table 1: Effect of Omeprazole on Food and Water Intakes in Male and Female Rats

Group	Male		Female		Combined	
	Food Intake (g)	Water intake (mL)	Food Intake (g)	Water intake(mL)	Food Intake (g)	Water intake(mL)
Control	190.04±7.29	74.75±7.750	190.66±7.62	67.29 ±7.77	190.40± 5.22	71.02 ± 5.45
OME 0.114 mg/kg	185.16±6.06	72.50±7.304	190.95±7.26	78.33 ±7.46	188.10 ± 4.69	75.42 ± 5.18
OME 0.57 mg/kg	190.25±7.63	80.21±8.212	184.12±7.09	69.79 ±8.69	187.20 ± 5.17	75.00 ± 5.96
OME 2.85 mg/kg	217.37±11.45***	78.13±7.438	203.37±8.51**	75.83 ±9.14	210.40±7.13****	76.98 ± 5.83

Values are mean ± SEM (n=6, 3 per sex). **P<0.01, ***P<0.001, ****P<0.0001 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole.

Table 2: Effect of Omeprazole on Biochemical Parameters in Male and Female Rats

Group	ALB (g/l)	GLU (mg/dl)	TP (g/l)	ALP (u/l)	ALT (u/l)	AST (u/l)	CREAT (mg/dl)	Urea (mg/dl)	BIL (mg/dl)
Control **Male**	36.53 ± 1.16	108.53 ± 2.02	38.40±6.38	56.03±11.8	37.60±7.20	40.40± 4.30	1.33± 0.33	44.96 ±3.78	0.77± 0.14
OME 0.114 mg/kg	39.13 ± 1.05	94.03 ± 2.28	40.73 ± 1.98	48.46 ±2.17	41.40±0.75	49.93±0.52	1.33 ± 0.16	49.86 ± 1.63	0.66±0.06
OME 0.57 mg/kg	39.96 ± 0.78	100.06 ± 4.86	45.33±1.56	51.76±11.91	44.93±2.69	46.26± 2.85	1.00 ±0.00	42.86 ± 3.27	0.67±0.06
OME 2.85 mg/kg	41.33 ± 2.72	104.63 ± 1.32	35.76 ± 3.77	55.53 ±9.89	33.90 ± 7.97	37.66 ± 12.34	1.33 ± 0.33	45.73 ± 4.04	0.80±0.11
Control **Female**	39.36±2.13	99.23±4.11	40.16±1.02	42.56±3.31	29.80±5.78	32.83±7.12	1.17±0.16	46.96±1.61	0.70±0.15
OME 0.114 mg/kg	39.66±1.73	104.66±1.35	41.23±5.92	59.23±10.37**	29.20±1.47	34.60±1.81	1.50±0.28	49.66±3.34	0.63±0.08
OME 0.57 mg/kg	40.10±2.40	100.76±4.32	34.00±4.31	51.60±6.27	37.93±4.22	42.60±4.23	1.00±0.00	38.50±3.35	0.53±0.03
OME 2.85 mg/kg	41.93±1.28	107.03±2.26	47.53±3.08	42.20±0.79	44.80±2.84*	52.23±1.24	1.66±0.16	45.00±4.50	1.10±0.20
Control **Combined**	37.95±1.25	103.88±2.92	39.28±2.92	49.30±6.26	33.70±4.48	36.61±4.08	1.250±0.17	45.97±1.89	0.73±0.09
OME 0.114 mg/kg	39.40±0.91	99.35±2.65	40.98±2.79	53.85±5.31	35.30±2.82	42.27±3.53	1.41±0.15	49.77±1.66	0.65±0.05
OME 0.57 mg/kg	40.03±1.13	100.41±2.91	39.66±3.26	51.68±6.02	41.43±2.73	44.43±2.42	1.00±0.00	40.68±2.31	0.60±0.04
OME 2.85 mg/kg	41.63±1.35	105.83±1.29	41.65±3.41	48.86±5.34	39.35±4.50	41.33±6.09	1.50±0.18	48.98±2.38	0.950±0.12

Values are mean± SEM (n=6, 3 per sex). *P<0.05, **P<0.01 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole; ALB: Albumin; GLU: Glucose; TP: Total Protein; ALP: Alkaline phosphatase; ALT: Alanine aminotransaminase; AST: Aspartate aminotransaminase; CREAT: Creatinine; BIL: Bilirubin.

Table 3: Effect of Omeprazole on Serum Electrolyte and Lipid Profile in Male and Female Rats

Group	CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	K (mg/dl)	Cl (mg/dl)	Na (mg/dl)
Male							
Control	212.66±24.65	158.10±9.84	56.90±0.30	114.16±13.21	3.67±0.08	98.76±1.23	145.86±3.80
OME 0.114 mg/kg	231.13±6.99	151.13±9.43	59.93±1.90	140.9±5.74*	3.40±0.09	101.86±1.06	144.36± 3.23
OME 0.57 mg/kg	217.90±12.74	151.16±6.40	61.73±1.62	125.93±13.12	3.60±0.05	99.40±2.21	139.73±3.29
OME 2.85 mg/kg	212.66±10.56	148.83±5.97	59.83±2.39	123.06±13.54	3.63±0.21	103.73±1.86	144.80±4.81
Female							
Control	207.30±7.03	155.50±3.35	62.00±2.11	115.66±6.67	3.53±0.20	100.03±1.06	140.37±4.56
OME 0.114 mg/kg	207.33±3.47	149.96±3.08	59.83±2.39	117.23±3.30	3.50±0.00	100.00±2.13	141.96±4.39
OME 0.57 mg/kg	216.60±10.32	154.60±4.17	60.13±1.48	125.53±9.70	3.53±0.20	100.00±2.84	143.06±3.73
OME 2.85 mg/kg	254.90±6.60****	155.77±7.19	63.07±0.53	159.97±7.73****	3.70±0.05	95.06±1.65	142.56±3.28
Combined							
Control	209.98±11.52	156.80±4.68	59.45±1.48	114.91±6.62	3.60 ±0.10	99.40±0.78	143.11±2.94
OME 0.114 mg/kg	219.23± 6.36	150.55±4.44	59.88±1.36	129.10±6.07	3.450±0.04	100.93±1.14	143.17±2.49
OME 0.57 mg/kg	217.25±7.34	152.88±3.50	60.93±1.04	125.73±7.30	3.56±0.09	99.70±1.62	141.40±2.35
OME 2.85 mg/kg	233.78±10.96**	152.30±4.46	61.45±1.31	141.51±10.80***	3.66±0.10	99.40±2.23	143.68±2.65

Values are mean± SEM (n=6, 3 per sex). *P<0.05,**P<0.01,***P<0.001,****P<0.0001 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole; CHOL: Cholesterol; TRIG: Triglycerides; HDL: High Density lipoprotein; LDL: Low Density lipoprotein; K: Potassium; Cl: Chloride; Na: Sodium.

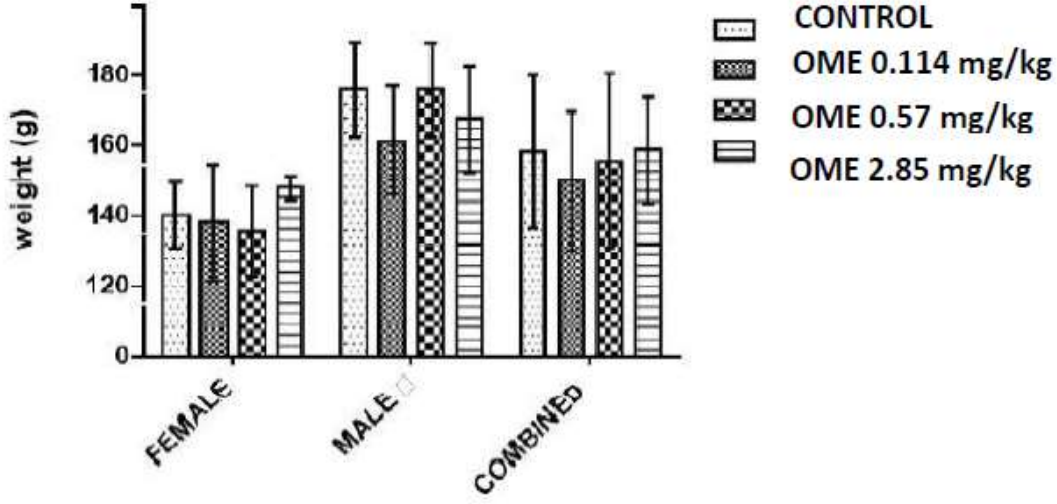


Figure 1: Effect of sub-chronic administration of Omeprazole on weight change in rats.
Values are mean ± SEM (n=6, 3 per sex). P>0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. Combined = Male + Female.

Table 4: Effect of Omeprazole on Haematological Parameters in Male and Female Rats

Group	WBC(10^3/µl)	LYM%	MID%	GRAN%	HGB (g/dl)	RBC (10^6/µl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	RDW-CV (%)
Male											
Control	9.20± 3.81	44.56±4.70	15.30±3.43	40.50±5.65	11.60±0.78	6.51±0.36	37.60±2.51	57.70±0.84	17.80±0.63	30.96±0.75	16.30±0.87
OME 0.114 mg/kg	8.43±0.46	36.63±10.44	30.06±15.39	31.30±4.02	13.63±0.38	9.30±1.80	43.53±1.08	59.76±2.57	18.63±0.49	31.26±0.70	15.46±0.26
OME 0.57 mg/kg	7.93± 0.91	51.16±5.90	16.43±0.75	28.16±5.70	13.76± 0.49	7.27±0.24	44.50±1.22	61.26±0.74	18.86±0.12	30.90±0.32	16.80±0.62
OME 2.85 mg/kg	12.20±1.15	52.13±1.30	16.20±2.45	31.66±1.81	12.40±1.60	6.42±0.70	39.76±4.93	61.80±1.58	19.16±0.54	31.06±0.20	16.30±0.58
Female											
Control	8.16±0.93	55.56±1.02	13.16±0.89	31.26±1.67	11.40±0.62	5.83±0.32	32.73±1.80	56.20±0.29	19.50±0.20	34.76±0.21	13.16±0.49
OME 0.114 mg/kg	9.40± 1.30	48.95 ± 6.45	15.65±5.15	34.70± 0.60	12.0 ± 0.93	6.81 ± 0.27	39.43±0.74	58.04±1.17	19.10 ±0.71	32.76 ± 0.82	14.20± 0.66
OME 0.57 mg/kg	10.80±0.11	54.96±0.92	10.60±1.58	33.26±1.33	13.03±0.35	6.56±0.10	38.80±0.23	59.20±0.59	19.80±0.23	33.53±0.71	13.76±0.82
OME 2.85 mg/kg	12.16±3.33	53.16±3.60	8.10±0.68	38.73±2.93	13.13±0.40	6.76±0.18	38.56±1.16	57.06±0.26	19.33±0.06	34.00±0.05	13.33±0.52
Combined											
Control	8.68±1.77	50.06±3.26	14.26±1.66	35.900±3.35	11.53±0.45	6.17±0.26	35.16±1.76	56.98±0.53	18.66±0.47	32.86±0.91	14.75±0.83
OME 0.114 mg/kg	8.82±0.53	41.56±6.78	24.30±9.28	32.66±2.36	12.85±0.57	8.05±0.98	41.48±1.08	58.90±1.32	18.90±0.40	32.01±0.59	14.83±0.42
OME 0.57 mg/kg	9.36±0.76	53.06±2.80	13.51±1.52	30.71±2.85	13.40±0.31	6.91±0.19	41.65±1.39	60.23±0.62	19.33±0.23	32.21±0.68	15.28±0.82
OME 2.85 mg/kg	12.18±1.57	52.65±1.73	12.15±2.13	35.20±2.20	12.76±0.75	6.59±0.33	39.16±2.28	59.43±1.27	19.25±0.24	32.53±0.66	14.81±0.75

	RDW-SD (fl)	PLT (10^4/µl)	MPV (FL)	PDW (%)	PCT (%)
Male					
Control	40.40±8.15	860.30±366.17	7.46± 0.47	15.43±0.35	0.44± 0.11
OME 0.114 mg/kg	31.16±1.15	823.66±2.05	7.16±0.21	15.30±0.15	0.62±0.04
OME 0.57 mg/kg	33.90±0.87	832.33±132.73	7.30±0.26	15.40±0.15	0.52± 0.04
OME 2.85 mg/kg	33.86±0.23	819.33±45.34	8.03±0.33	15.30±0.05	0.56±0.02
Female					
Control	26.90±1.04	701.33±63.19	7.13± 0.17	15.06±0.06	0.50± 0.05
OME 0.114 mg/kg	28.63± 0.64	756.66± 50.07	7.20 ±0.20	15.20 ± 0.17	0.54±0.02
OME 0.57 mg/kg	29.20±1.00	768.33±84.30**	7.36±0.38	15.13±0.08	0.56±0.07
OME 2.85 mg/kg	27.66±0.64	806.33±18.22**	7.00±0.11	14.93±0.08	0.49±0.03
Combined					
Control	33.66±4.76	780.83±169.93	7.30±0.23	15.25±0.18	0.47±0.05
OME 0.114 mg/kg	31.55±1.20	800.33±71.76	7.33±0.20	15.26±0.09	0.54±0.03
OME 0.57 mg/kg	29.90±0.82	790.16±30.51	7.18±0.13	15.25±0.10	0.58±0.03
OME 2.85 mg/kg	30.76±1.42	812.83±83.83	7.51±0.27	15.11±0.09	0.53±0.02

Values are mean ± SEM (n=6, 3 per sex). **P<0.01 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole; WBC: White Blood Cells; LYM%: Lymphocytes differentials; MID%: Differentials of the other types of white blood cells not classified as lymphocytes or granulocytes; GRAN%: Granulocyte differentials; HGB: Hemoglobin; RBC: Red Blood cell; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; PLT: Platelet; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Plateletcrit.

Table 5: Effect of Omeprazole on Serum Antioxidants in Male and Female Rats

Group	GSH (µmol/ml/mg protein)	SOD (µmol/ml/mg protein)	CAT (µmol/ml/mg protein)	MDA (µmol/ml/mg protein)
Male				
Control	0.62±0.18	3.71±0.97	16.17±4.97	0.04±0.01
OME 0.114 mg/kg	0.44±0.07	2.92±0.22	13.34±1.27	0.05±0.00
OME 0.57 mg/kg	0.27±0.08	2.59±0.43	9.66±2.17	0.05±0.01
OME 2.85 mg/kg	0.33±0.06	2.71±0.37	8.95±1.43	0.04±0.00
Female				
Control	1.08±0.18	3.28±0.73	19.45±6.23	0.09±0.03
OME 0.114 mg/kg	1.42±0.39	2.51±0.22	9.90±1.39*	0.05±0.03
OME 0.57 mg/kg	1.65±0.72	3.96±1.82	15.07±12.45	0.08±0.04
OME 2.85 mg/kg	1.15±0.05	2.46±0.13	15.86±1.58	0.07±0.02
Combined				
Control	0.85±0.15	3.50±0.55	17.81±3.64	0.06±0.01
OME 0.114 mg/kg	0.93±0.28	2.72±0.16	11.62±1.14*	0.05±0.01
OME 0.57 mg/kg	0.96±0.45	3.28±0.89	12.37±5.78	0.06±0.02
OME 2.85 mg/kg	0.74±0.18	2.58±0.18	12.40±1.81	0.06±0.01

Values are mean ± SEM (n=6, 3 per sex). *P<0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole; GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

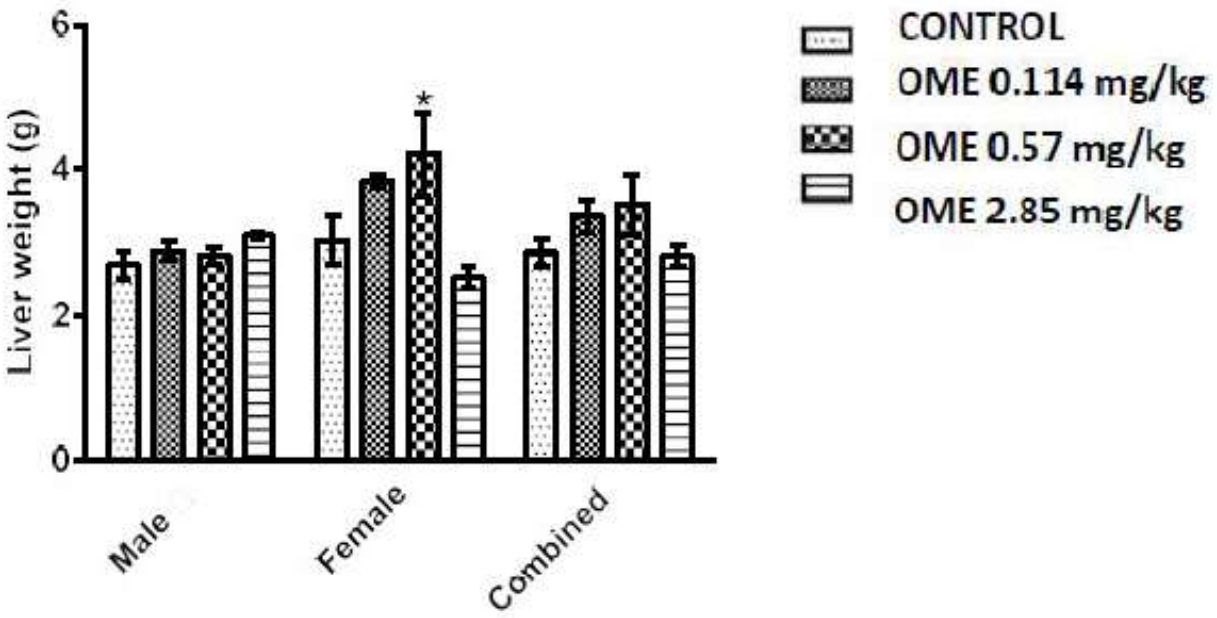


Figure 2: Effect of sub-chronic Omeprazole administration on rats' liver weight.
Values are mean ± SEM (n=6, 3 per sex). *P< 0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. Combined = Male + Female.

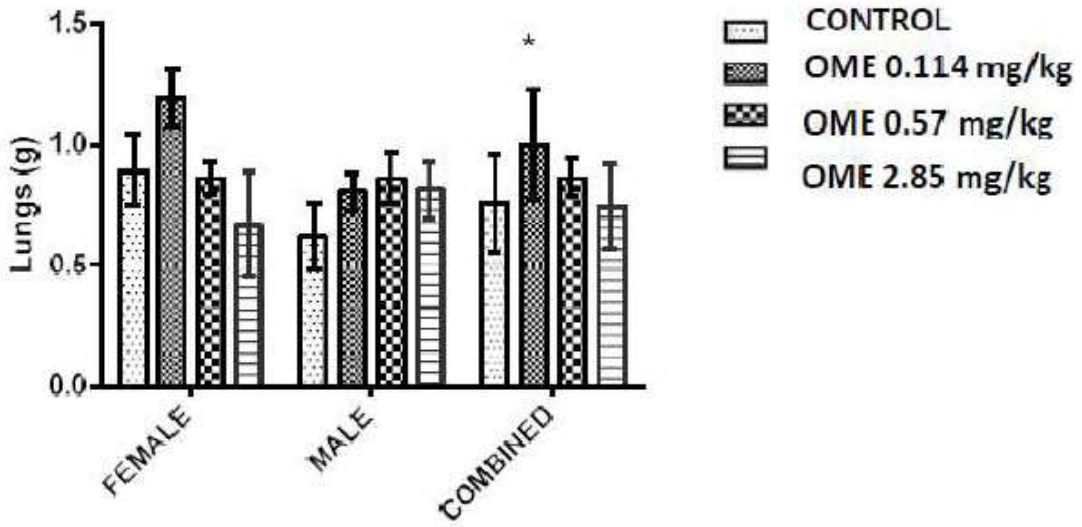


Figure 3: Effect of sub-chronic Omeprazole administration on rats' lungs weight.
Values are mean ± SEM (n=6, 3 per sex). *P<0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. Combined = Male + Female.

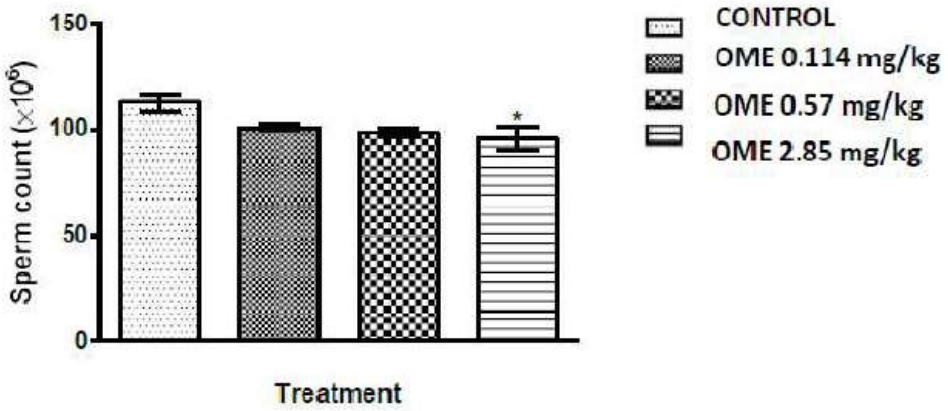


Figure 4: Effect of sub-chronic Omeprazole administration on sperm count in rats.
Values are mean ± SEM (n=3). *P<0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole.

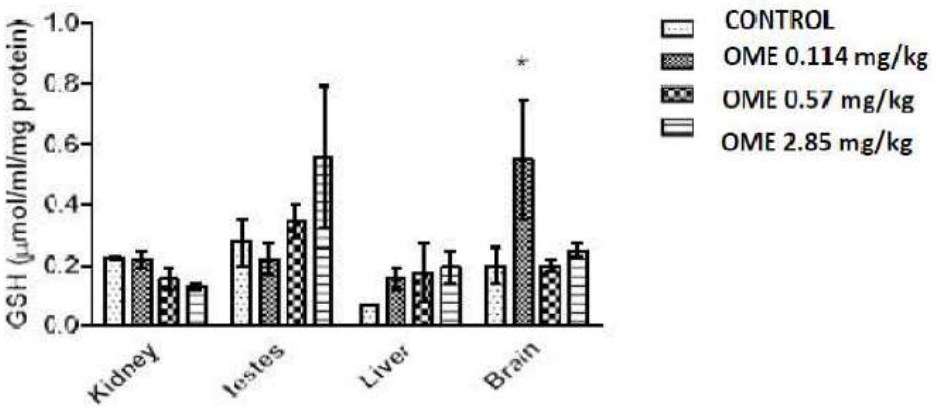


Figure 5: Effect of sub-chronic Omeprazole administration on GSH level in male rats.
Values are mean ± SEM (n=3). *P<0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. GSH: Reduced glutathione.

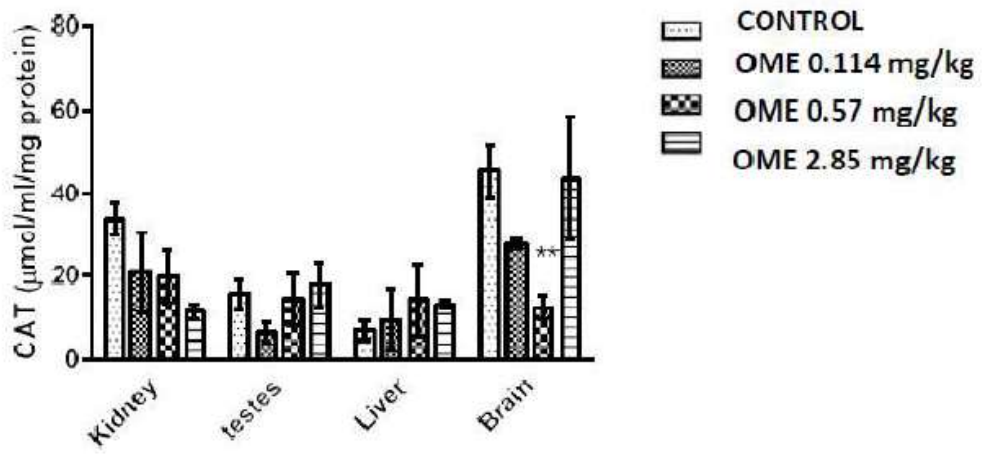


Figure 6: Effect of sub-chronic Omeprazole administration on CAT level in male rats.
Values are mean ± SEM (n=3). **P<0.01 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. CAT: Catalase.

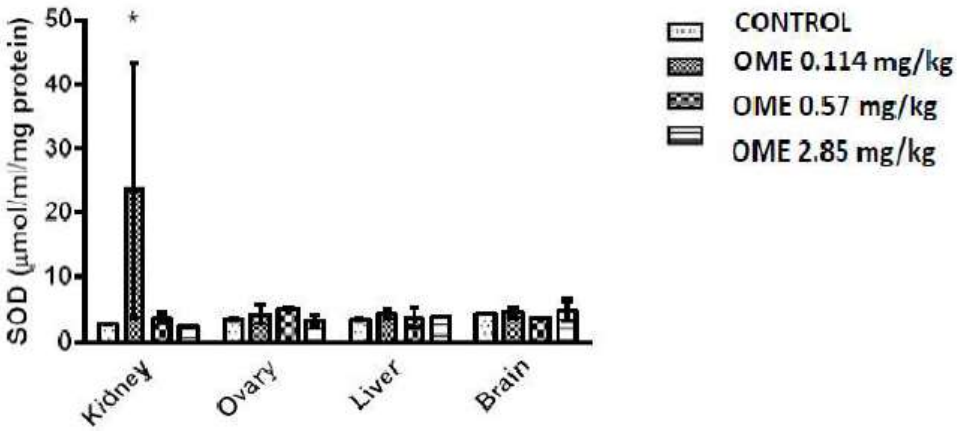


Figure 7: Effect of sub-chronic Omeprazole administration on SOD level in female rats.
Values are mean ± SEM (n=3). *P<0.05 vs. control (one-way ANOVA followed by Dunnett's multiple comparison test). OME: Omeprazole. SOD: Superoxide dismutase.

4.0 Discussion

There are two main positions on the safety of the long-term use of OME in both academic and medical circles. One position believes that there are no safety issues to be concerned about (Attwood *et al*., 2015), while the other position believes that there are indeed safety issues (Abbas *et al*., 2019; Paz *et al*., 2020) with long-term use of OME. This study investigated the effect of 90 days sub-chronic administration of OME on haematological, biochemical, antioxidant, reproductive and histopathological parameters in male and female rats. The overall objective of this study is to contribute to the academic and medical discussion on the overall safety of the long-term use of OME based on recent warnings issued by the United States FDA on its use.

In this study, the administration of OME did not lead to any significant reduction in the body weight, weight of vital internal organs, and food and water intakes of test animals. Significant reduction in body weight or vital organ weight is known to be associated with toxicity (Raza *et al*., 2002; Tan *et al*., 2008). However, it is important to note that the supratherapeutic dose of OME elicited a non-significant decrease in weight of the liver in female animals. The non-significant impact of OME on body weight, weight of vital organs, and food and water intakes in this study suggest safety of the drug. After 90 days administration of OME, a significant reduction in sperm count was observed at the supratherapeutic dose. This seems to suggest that long-term administration of OME at higher doses may potentially lead to negative changes in sperm characteristics.

It is important to note, however, that there was no significant change in other sperm characteristics (motility and morphology) in this study. Banihani (2016) in his mini-review paper on "Omeprazole and semen quality" suggested that OME does not appear to change semen quality or worsen sperm condition citing several seminal works in literature. Based on this, we suggest more research to confirm this finding on the negative effect of OME on sperm characteristics observed in this study. Additionally, antioxidant parameters observed in the testes in this study were generally non-significant and cannot be directly extrapolated to have a negative impact on sperm cells.

OME has been reported to increase oxidative stress (Herbet et al., 2016) leading to DNA changes, possibly through the generation of excessive ROS (Vidya et al., 2015). This is in line with similar findings in which the co-administration of OME with antioxidant agents

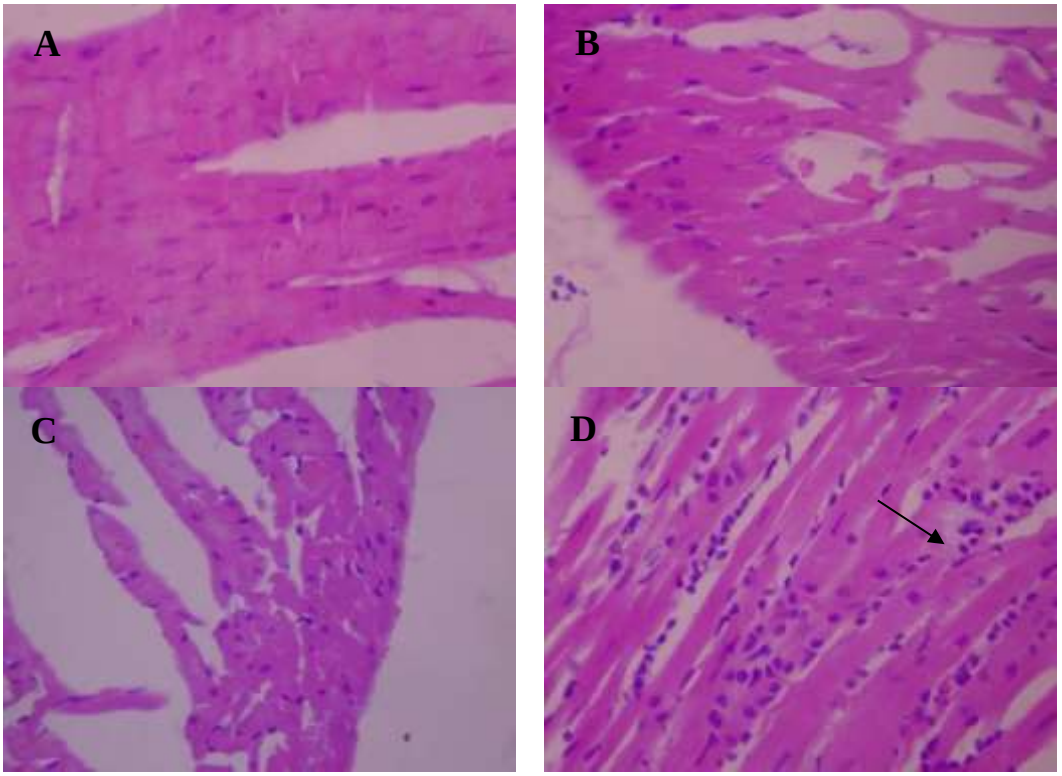


Figure 8: Heart histological photomicrographs of female rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at doses of 0.114 mg/kg (B), 0.57 mg/kg (C), and 2.85 mg/kg (D) for 90 days. A - C shows no abnormalities, while D shows myocardial inflammation.

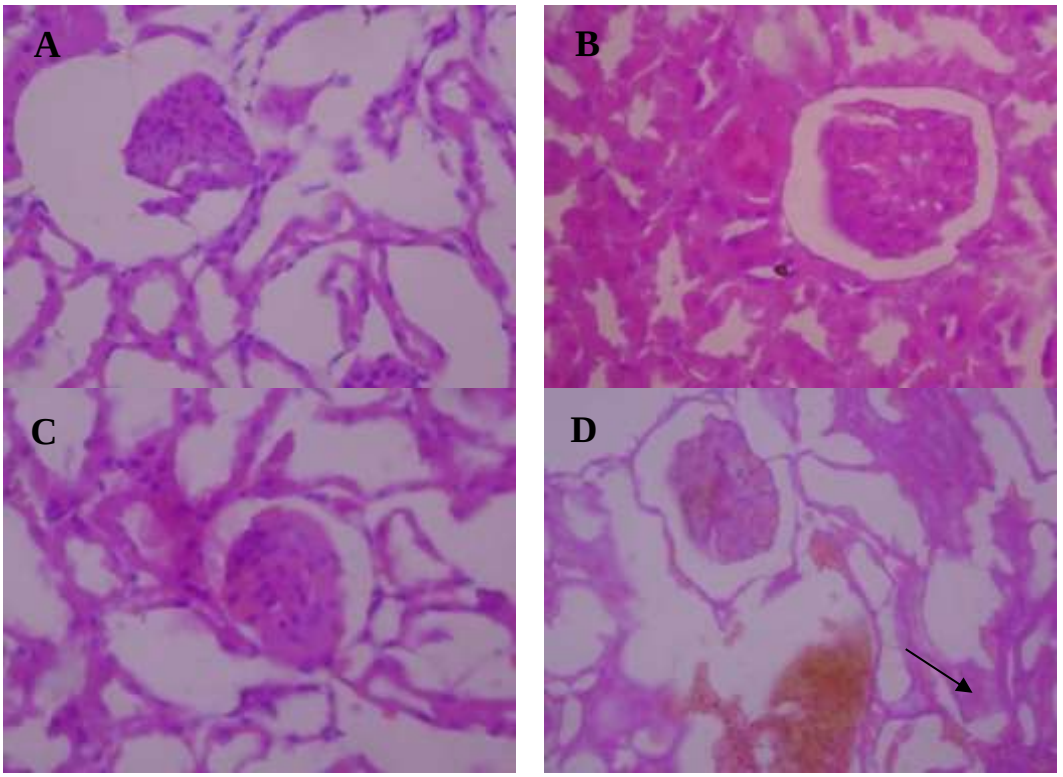


Figure 9: Kidney histological photomicrographs of female rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at doses of 0.114 mg/kg (B), 0.57 mg/kg (C), and 2.85 mg/kg (D) for 90 days. A - C shows no abnormalities while D shows vascular congestion.

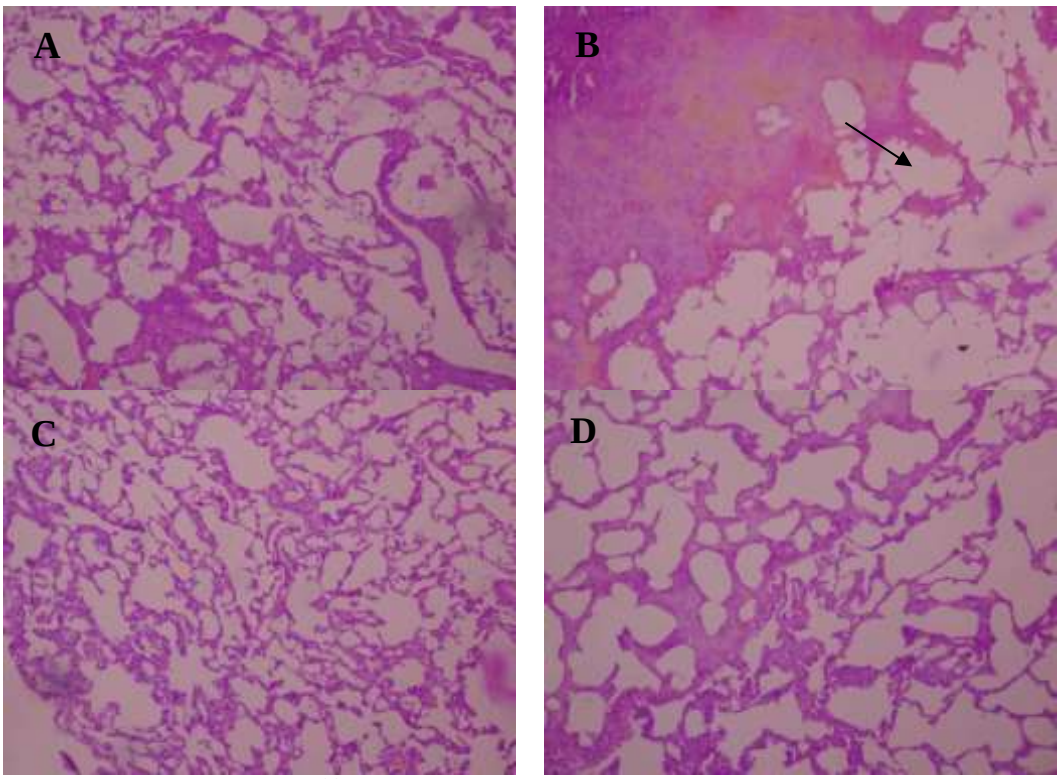


Figure 10: Lungs histological photomicrographs of female rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at doses of 0.114 mg/kg (B), 0.57 mg/kg (C), and 2.85 mg/kg (D) for 90 days. A, C and D show no abnormalities, while B show moderate pulmonary inflammation.

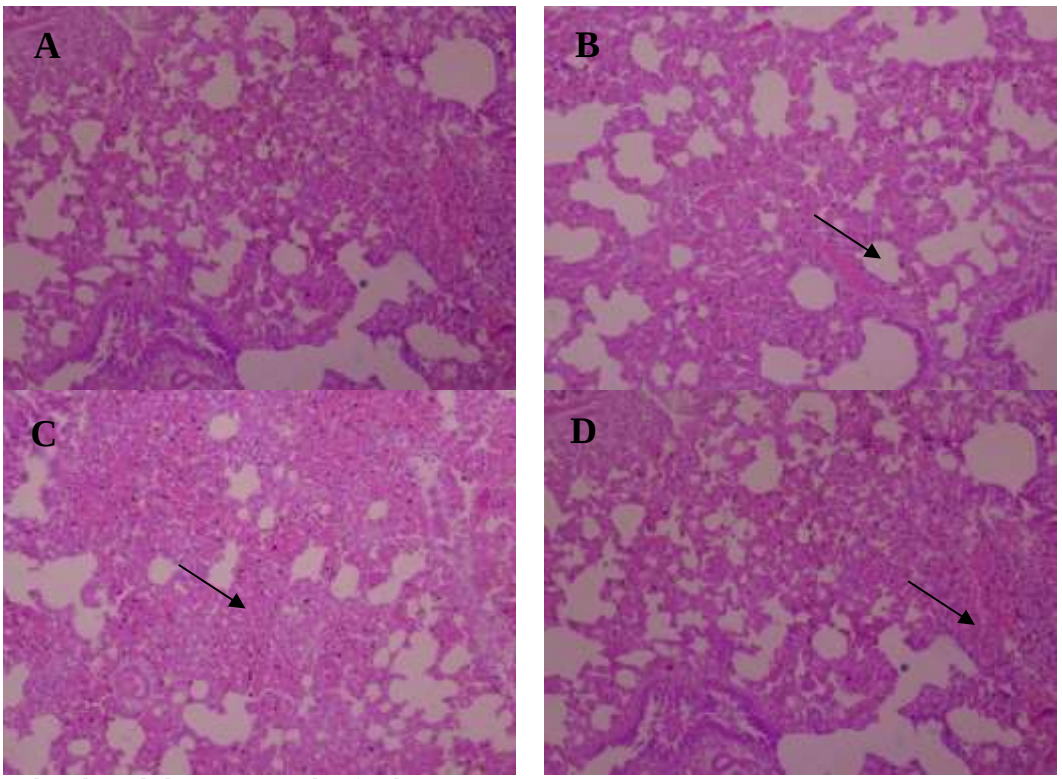


Figure 11: Lungs histological photomicrographs of male rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at doses of 0.114 mg/kg (B), 0.57 mg/kg (C), and 2.85 mg/kg (D) for 90 days. A show no abnormalities while B, C and D show moderate to severe pulmonary inflammation.

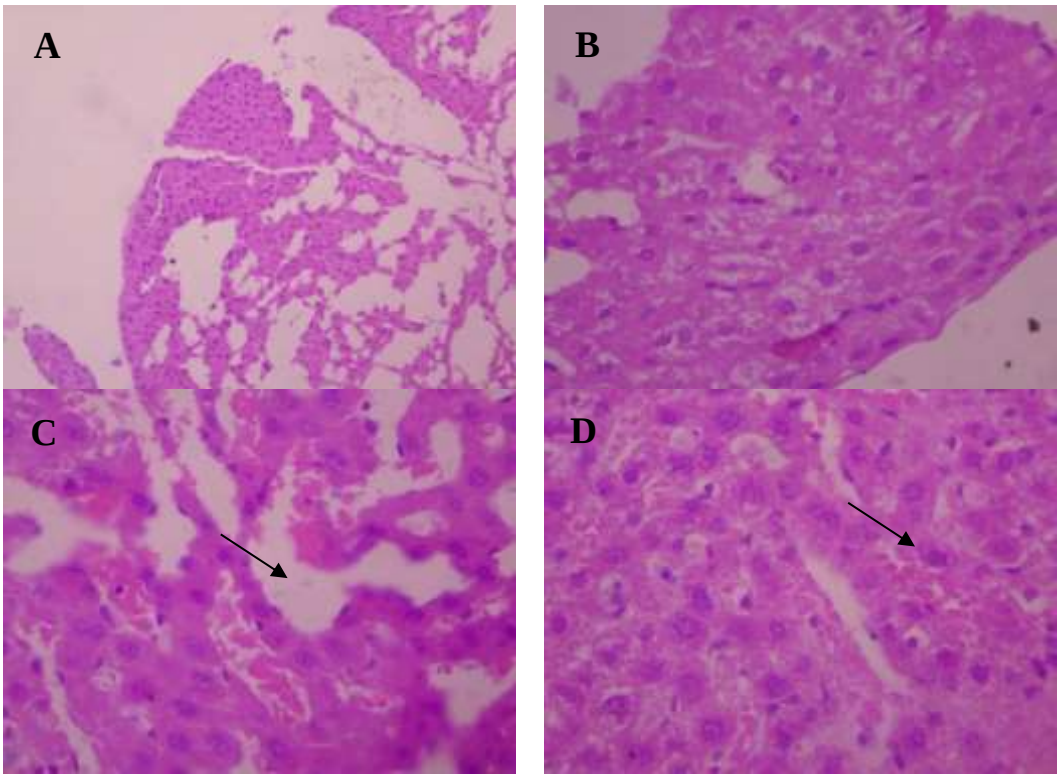


Figure 12: Liver histological photomicrographs of female rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at doses of 0.114 mg/kg (B), 0.57 mg/kg (C), and 2.85 mg/kg (D) for 90 days. A and B show no abnormalities, while C and D show sinusoidal congestion.

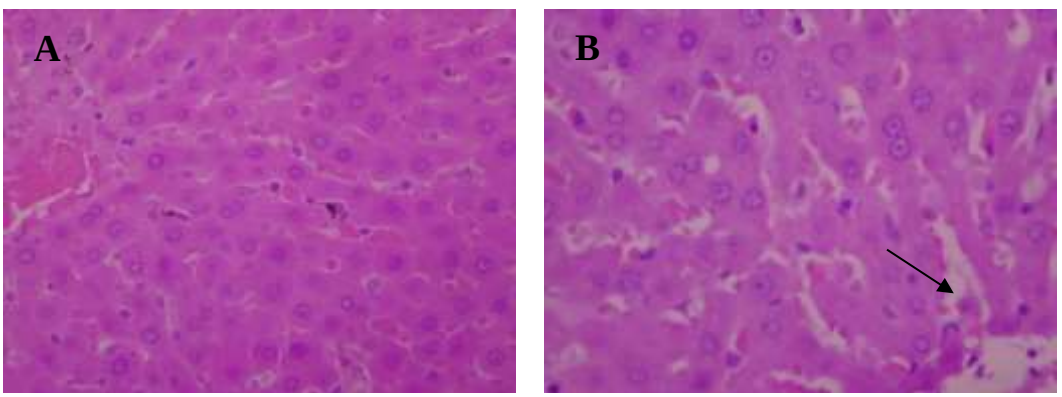


Figure 13: Liver histological photomicrographs of male rats.
Shows representative photomicrographs for groups orally dosed with distilled water (A) or Omeprazole at dose of 2.85 mg/kg (B) for 90 days. A show no abnormalities, while B show sinusoidal congestion.

(e.g. vitamin E) reversed the oxidative stress effect of OME and inhibited its genotoxic and mutagenic effects (Guduru and Rohit, 2016; Braga et al., 2018, 2021; Paz et al., 2020). Conversely, OME has been reported to increase in-vivo antioxidant levels and capacity (Koch et al., 2002; Biswas et al., 2003; Ganguly et al., 2006; Al-Sayed et al., 2015). This suggests a contradictory antioxidant effect of OME as reported in literature. In this study, OME elicited a significant reduction in blood levels of catalase and a non-significant reduction in other antioxidant parameters like GSH and SOD, alluding to the oxidative stress potential of OME. This is one of the mechanisms of the adverse effects of long-term use of OME (Paz et al., 2020). In the tissues, there was a general non-significant change in antioxidant parameters. However, there was a significant elevation of SOD and GSH levels in the kidney and brain respectively, suggesting possible OME antioxidant effect. At the same time, there was a non-significant elevation in MDA level in the kidney and significant reduction in CAT level in the brain, suggesting possible oxidative stress effect of OME. These results show contradictory effects of OME, at the same time making it difficult to make direct extrapolations of effects. It is worthy to mention that the antioxidant effects were generally observed at the sub-therapeutic dose (0.114 mg/kg) of OME, while the oxidative stress effects were generally observed at higher doses in the tissues. In the blood, the oxidative stress capacity of OME was observed across all doses. We recommend that more research be conducted on the antioxidant or oxidative stress mechanism of OME to elucidate this contradictory effect. From our study, it is quite difficult to make any valid extrapolations or suggest any variations based on the different sexes of test animals.

The increase in the levels of ALP and ALT is somewhat indicative of the presence of liver damage and is an important premise to consider in toxicological studies as the liver is one of the most important organs in the human body. In this study, OME significantly increased the levels of ALP at the sub-therapeutic dose and ALT at the supratherapeutic dose in female animals. There was a corresponding non-significant change in male animals suggesting some variations in effect between female and male animals. While ALT is a more specific indicator of liver function as it is found mainly in the liver, ALP is also indicative of liver function but is found in other tissues like bone, placenta, kidney, and gut. Hence, ALP may be raised in the presence of normal liver health. In this study, ALP was raised at the sub-therapeutic dose which makes it difficult to make a conclusive extrapolation of observed effects. On the other hand, ALT values were elevated significantly. While this is more indicative of liver damage, the elevation of ALT at the supratherapeutic dose is less than a 2-fold rise from the control value. This again makes it difficult to make a conclusive extrapolation of observed effects. However, histopathological assessment of the liver showed sinusoidal congestion at both therapeutic and supratherapeutic doses of OME in female animals, suggesting a negative effect of long-term use of OME in the liver.

While we do not and cannot categorically connect long-term use of OME with liver damage based on findings in this study, we believe that this is an area that requires further research. It is important to note that OME has been reported to lead to liver damage (Fatima et al., 2019), show a negative effect on hepatic regeneration (Kucuk et al., 2006) and produce elevated levels of GPT and GOT liver enzymes which are indicative of liver dysfunction (Jassim and Nasser, 2018). Additionally, omeprazole was reported to induce increases in AST, ALT, and cholesterol which decreased after cessation of treatment (Elmahdy and Almater, 2019). Furthermore, AST/ALT ratio in this study was greater than 1 suggesting that the liver damage may be due to liver cirrhosis.

One of the functions of the liver is the breakdown of excess cholesterol and the prevention of build-up of cholesterol in the body (Trefts et al., 2017; Kalra et al., 2018). As a result, when there is liver damage or cirrhosis, there is a possibility of build-up of cholesterol leading to high cholesterol levels in the body system. In this study, long-term administration of OME produced a significant increase in the levels of cholesterol and low-density lipoproteins (LDL) in the male and female animals. This suggests that long-term administration of OME may potentially lead to liver damage as evidenced by high levels of ALT, cholesterol, and LDL observed in this study. Histopathological assessment of the heart shows myocardial inflammation in female animals at higher doses, potentially due to high cholesterol (hypercholesteremia) and high lipid levels (hyperlipidaemia).

Results from the 14-year Safety of Omeprazole in Peptic Reflux Esophagitis: A Nordic Open Study (SOPRAN) indicated that no clinically relevant changes in haematological parameters were observed over the extensive study period (Attwood et al., 2015). The findings from the SOPRAN study are consistent with the findings in this study in which no significant change in haematological parameters was observed, except for a significant increase in platelet levels in female animals at higher doses. There was a non-significant change in platelet levels in male animals. An increase in platelet level above normal is known to be associated with either of two conditions called primary or essential thrombocythemia or secondary thrombocytosis (Skoda, 2009; Robinson and Godfrey, 2021). Primary or essential thrombocythemia occurs when faulty stem cells in the bone marrow allow too many platelets to be produced (Robinson and Godfrey, 2021). The cause of this is usually not known. Secondary thrombocytosis occurs if there is an underlying disease or condition responsible for the higher-than-normal platelet level (Skoda, 2009). Such underlying disease or condition could be a malignancy, an infection, chronic inflammation, trauma, surgery, iron deficiency or splenectomy (Voutsadakis, 2014). However, the common premise for all these conditions is inflammation (Ertenliet al., 2003) involving inflammatory cytokines which are able to stimulate the process of platelet production (Voutsadakis, 2014) under inflammation. Furthermore, an increase in platelet count has been reported to be associated with an increased risk of cancer and platelets are known to play a major role in carcinogenesis and cancer progression (Giannakeas and Narod, 2021). Additionally, cancer is a pathology that is often associated with thrombocytosis and thrombocytosis is a known universal marker of adverse outcomes in cancer (Voutsadakis, 2014). Based on all of these, it can be inferred that long-term use of OME at high doses is potentially carcinogenic and thrombocytosic. Indeed, the genotoxic, mutagenic, and carcinogenic effects of OME is well documented in literature (Rosenkranz and Klopman, 1991; Jyothsna and Rohit, 2016; Braga et al., 2018; Paz et al., 2020). This corroborates the findings in this study. Additionally, chronic use of OME has been associated with an increased risk of gastric cancer (Abbas et al., 2019; Paz et al., 2020). It can therefore be theorized that long-term use of OME, especially at high doses, may increase the incidence of cancer especially in female animals. We recommend more research to confirm these findings, especially the variation of effects in different sexes.

5.0 Conclusion

From the findings in this study, it can be concluded that the administration of OME over a long time is generally safe and tolerable, especially at lower doses. However, results suggest that OME may potentially induce changes in sperm characteristics, hepatoxicity, hypercholesteremia, hyperlipidemia and thrombocytosis at higher doses and over a long duration. It is advised that physicians consider these potential effects while prescribing OME, especially for long-term administration. Additionally, this study provided insights into the possible variation in response to OME between male and female animals. Female animals seem to be more affected by the adverse effects of long-term use of OME than male animals. This potentially has important implication in the prescription of OME to female patients. It might be important that dose reduction or adjustment be considered when long-term use of OME is being prescribed to female patients. Further research to explore and confirm these variations in observed effects in male and female animals is highly recommended. One limitation of this study is the absence of reversibility studies to evaluate the potential for reversal of observed effects after cessation of OME administration.

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Conflict of interest

The authors have no conflict of interest relating to this study to declare.

Author's contribution

Conception: AJA
Design: AJA
Execution: AEO and AOI
Interpretation: AJA, AEO, FJO and AOI
Writing the paper: AJA, AEO, FJO and AOI

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