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Effects of selected oral contraceptives on the kidney functionality

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Abstract

Overtime, oral contraceptives has been used as an option for prevention of pregnancy due to their synthetic hormones composition that prevents fertilization. Regardless of their usefulness in contraception, studies have shown that when these drugs are abused, they could cause some adverse effects in the normal body metabolism. This study investigated the effects of Exluton and Combination-3 on serum Electrolyte (Sodium; Na^+, Potassium; K^+, Chloride; Cl^-, and Bicarbonate; HCO_3^-), Urea and Creatinine in female wistar rats as an indications of kidney function. The study was carried out using twenty-five female albino rats which was grouped into five groups of five rats each. Group one was the control group, group two was administered with Low Dose Exluton, group three High Dose Exluton, group four Low Dose combination-3 and group five High Dose Combination-3. After a period of 21 days' administration, the blood samples of the experimental rats were collected for bioassay of the kidney function while the kidney harvested for histological examination. The results from the analysis revealed that on the administration of both drugs, a significant increase at p value 0.05 was observed in the serum concentrations of Urea, Creatinine, Na^+ and K^+. The increase was most in the groups administered Combination-3 when compared to the control group. A decrease in the serum concentration of Cl^- and HCO_3^- was observed when compared to the group. However, an increase in Cl^- and HCO_3^- concentrations were observed in the group administered low dose Exluton, but not significant at 0.05 level. The findings of this study therefore suggests that both contraceptives of study may pose the tendency of kidney toxicity when abused, with greater tendency in the groups administered Combination-3.

Keywords: Exluton; Combination-3; Kidney; Serum Electrolytes; Urea; Creatinine

1. Introduction

Toxicity of the kidneys could be attributed to several factors, it can also be attitudinal, such as abuse of drugs [1] and negligence concerning one's health. Urea and Creatinine are good indicators of a normal functioning kidney and increase in their serum concentrations are indications of kidney dysfunction [2].

Creatinine, is a non-protein nitrogenous (NPN) waste product, gotten from the breakdown of Creatinine and phosphocreatine and can serve as an indicator of renal function [3]. It is a by-product of muscle protein metabolism and an increase in the Creatinine level usually indicates renal failure or massive muscle damage. It is produced endogenously within the body and is freely filtered by the glomerulus; this therefore makes it a useful endogenous marker for creatinine clearance [4]. The concentration of plasma creatinine and the estimated glomerular filtration rate calculated from plasma creatinine, age, weight, and gender, are used to assess kidney function [5].

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Urea is also a metabolic by-product of the breakdown of amino acids and is excreted by the kidneys [5] which can build up if kidney function is impaired. The BUN-to-Creatinine ratio generally provides more precise information about kidney function and its possible underlying cause compared with creatinine level alone [6].

Sodium (Na+) and Potassium (K+) are electrolytes charged atoms (ions) that allow passage of charges. They are written with a small plus or minus, indicating their electrical charge. Bicarbonate (HCO3-) is important in determining the pH of the blood, indicating acidosis and alkalosis. The pH is defined by hydrogen ion (H+) levels [7]. Potassium is the ion most present in body fluids and approximately, 98% of its concentration is intracellular. The main physiological functions of the ion are to adjust the cellular metabolism such as the protein and glycogen synthesis, and the basic acid balance [8].

Lynestrenol, sold under the brand names Exluton and Ministat among others, is a progestin medication which is used for treatment of gynecological disorders and in prevention of pregnancy [9].

Combination-3 is a contraceptive drug made up of Progestin (synthetic progesterone) and Estrogen which helps in prevention of pregnancy [10].

Amidst the numerous benefits of these drugs in contraception, researchers have reported its possible alteration of the metabolism of the biological system when abused or taken in doses not prescribed doses[11], hence the need to carry out this study due to the administration of oral contraceptives in doses not prescribed in recent times. This study investigated the effects of Exluton and Combination-3 on serum Electrolyte (Sodium; Na+, Potassium; K+, Chloride; Cl-, and Bicarbonate; HCO3-), Urea and Creatinine in female wistar rats as an indications of kidney function.

2. Material and methods

2.1. Experimental animals

Twenty-five adult female albino rats were obtained from the Animal house of Rivers State University, Port Harcourt and taken to the experimental laboratory where they were divided into 5 groups of five albino rats each. They were allowed standard feed and water *ad libitum* and allowed to acclimatize for 21 days.

2.2. Drug of study

Exluton is a brand of progesterone only pill containing specifically 500mirogram of lynestenol in each tablet. A combination 3 pills is a COCP containing levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg and iron based compound, iron fumarate.

Both drugs were purchased from a Medical Pharmacy in Port Harcourt, Rivers State, Nigeria. The Combination 3 tablet used consists of 21 hormonal tablets and 7 non-hormonal tablets. Each white hormonal tablet contains low doses of estrogen and progesterone hormones.

2.3. Experimental grouping/drug administration

The female albino rats were divided into five groups:

- Group 1: Feed + Water only
- Group 2: Low dose Exluton + feed + water
- Group 3: High dose Exluton + Feed + Water
- Group 4: Low dose Combination 3 + Feed + Water
- Group 5: High dose Combination 3+ Feed + Water

Administration of drugs were as reported Tietz, (1994), Each day a tablet is dissolved in 100 mL distilled water and the appropriate dose per kg was measured out using a 2 mL syringe for oral administration via an oro-gastric tube. Low dose received 0.14 mL while high dose received 0.30 mL of the prepared drug. These doses were determined based on comparative dosage per body weight proportion akin to humans.

2.4. Sample collection

Twenty-four hours after the last administration, the albino rats were sacrificed, blood samples were collected from each of the rats into sterile sample bottles for analysis of the kidney function biomarkers and the Kidneys of each wistar rat was harvested for histological examination.

2.5. Sample analysis

Kidney function was evaluated using the serum. Urea and Creatinine was analyzed using the method as described by Zhang et al., (2017). Electrolytes were analyzed using the method described by Kim et al., (2018).

2.6. Histological examination of the kidney

Kidneys were washed with saline to remove bloodstain and fixed in Bouin's fixative, dehydrated with different grades of alcohol, cleared in chloroform, infiltrated with molten paraffin wax and embedded in paraffin wax. Sections of 5µm thickness were taken and stained with haematoxylin and eosin and evaluated under the light microscope (Odinga et al., 2022; Bancroft and Gamble, 2006).

2.7. Data analysis

The Mean ± Standard deviation was determined using one-way analysis of variance (ANOVA) and the Turkey Post Hoc test was done for multiple comparisons. The significance level was set at $p < 0.05$.

3. Results

Table 1 Concentration of Urea and Creatinine in experimental rats

Groups	Urea (mmol/l)	Creatinine (µmol/l)
Control	$5.13^{a} \pm 0.59$	$103.00^{a} \pm 12.62$
LD Exluton	$9.62^{b} \pm 1.02$	$181.25^{b} \pm 12.28$
HD Exluton	$8.87^{bc} \pm 0.27$	$173.50^{b} \pm 7.32$
LD Combi-3	$9.86^{bcd} \pm 0.33$	$195.00^{bd} \pm 12.54$
HD Combi-3	$8.60^{bcde} \pm 1.01$	$164.75^{bce} \pm 14.38$

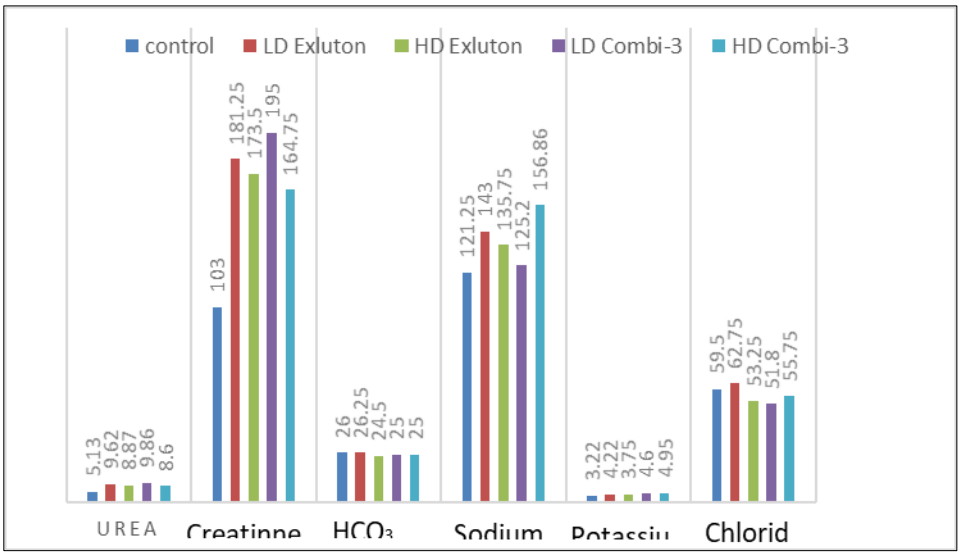
Values are Mean ± Standard deviation with significance level measured at $p<0.05$. Values with the same superscript shows no significance at 0.05, Values with different superscripts shows significance at 0.05, LD: Low Dose, HD: High Dose

Table 2 Concentration of Serum Electrolytes in experimental rats

Groups	HCO3-(mmol/l)	Na+(mmol/l)	K+(mmol/l)	Cl-(mmol/l)
Control	$26.00^{a} \pm 1.82$	$121.25^{a} \pm 16.17$	$3.22^{a} \pm 0.45$	$59.50^{a} \pm 6.55$
LD Exluton	$26.25^{a} \pm 3.30$	$143.00^{a} \pm 5.75$	$4.22^{a} \pm 0.45$	$62.75^{b} \pm 13.81$
HD Exluton	$24.5^{a} \pm 3.1$	$135.75^{a} \pm 6.75$	$3.75^{ac} \pm 0.28$	$53.25^{a} \pm 1.70$
LD Combi-3	$25.00^{a} \pm 3.60$	$131.20^{a} \pm 16.79$	$4.60^{ad} \pm 0.71$	$51.80^{a} \pm 3.40$
HD Combi-3	$25.00^{a} \pm 2.76$	$156.8^{e} \pm 10.33$	$4.95^{e} \pm 0.34$	$55.75^{a} \pm 3.50$

Values are Mean ± Standard deviation with significance level measured at $p<0.05$. Values with the same superscript a as reflected in the control group shows no significance at $p< 0.05$, Values with different superscripts ac or ad as noted for HD Exluton,LD Combi-3 groups shows significance at $p< 0.05$, LD: Low Dose, HD: High Dose

The results on table 1& 2 for the effect of Exluton and Combination-3 on the Serum urea, creatinine and Electrolytes as indication of kidney function revealed that both oral contraceptives at the various doses administered significantly caused an increased variation at p value 0.05 in the concentration of the serum Urea, Creatinine, and potassium biomarkers, although the levels of Chloride and Bicarbonate were significantly decreased except in Low dose of Exluton at P value 0.05 level.



x- axis: Control – Normal control group; LD: low dose; HD: high dose; HCO_3^-: Bicarbonate; Combi-3: Combination-3

Figure 1 Mean concentrations of Urea, Creatinine and Serum Electrolytes of experimental rats

3.1. Histological examination of the kidney

The histological examination of the kidney of the wistar rats of study were as follows:

Group 1 (Control) revealed the renal tissues with normal tubules and normal Glomeruli; LD Exluton(group 2) showed normal tubules and mild mesangial proliferation of the glomerulus; HD Exluton(group 3) showed mild proliferation, patchy necrosis, perinuclear clearing of the epithelial cells lining the tubules hence suggesting fatty infiltration; LD Combi-3 (Group 4) revealed mesangial proliferation and fibrosis with normal tubules and the renal tissues, HD combi-3(group 5) also revealed mesangial proliferation and fibrosis with normal tubules.

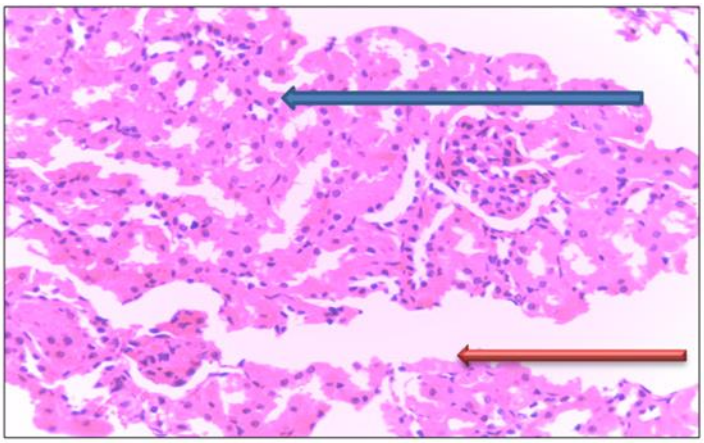


Figure 2 Section of kidney from the Control group showing normal tubules (red) and normal glomeruli (blue) (H&E ×400)

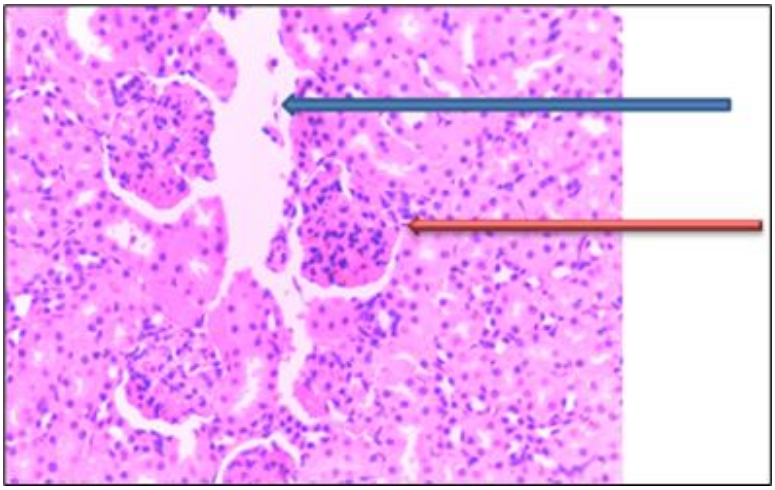


Figure 3 Section of kidney from group 2 showing normal tubules (red) and mild mesangial proliferation of the glomerulus (blue) (H&E ×400)

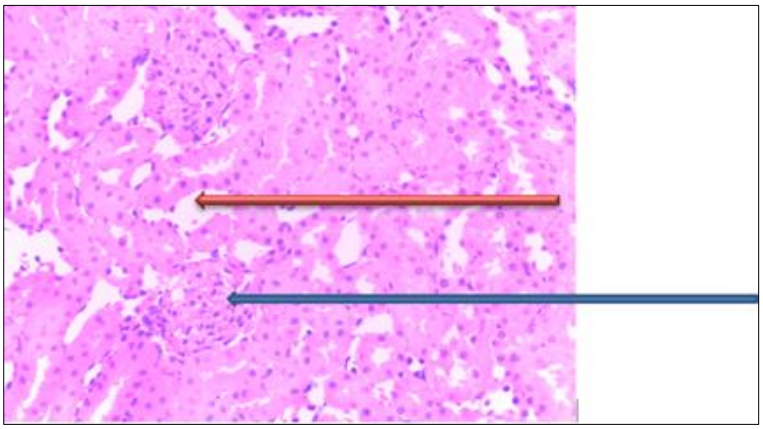


Figure 4 Section of kidney from Group 3 showing mild mesangial proliferation (blue) and patchy necrosis of the renal tubules(red) (H&E ×400)

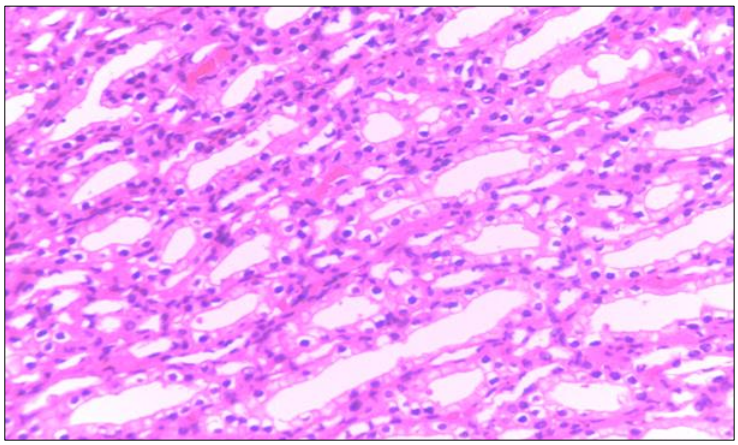


Figure 5 Section of kidney from group 4 showing mesangial proliferation and fibrosis with normal tubules and the renal tissues, also showing perinuclear clearing of the epithelial cells lining the tubules suggestive of fatty infiltration (H&E ×400)

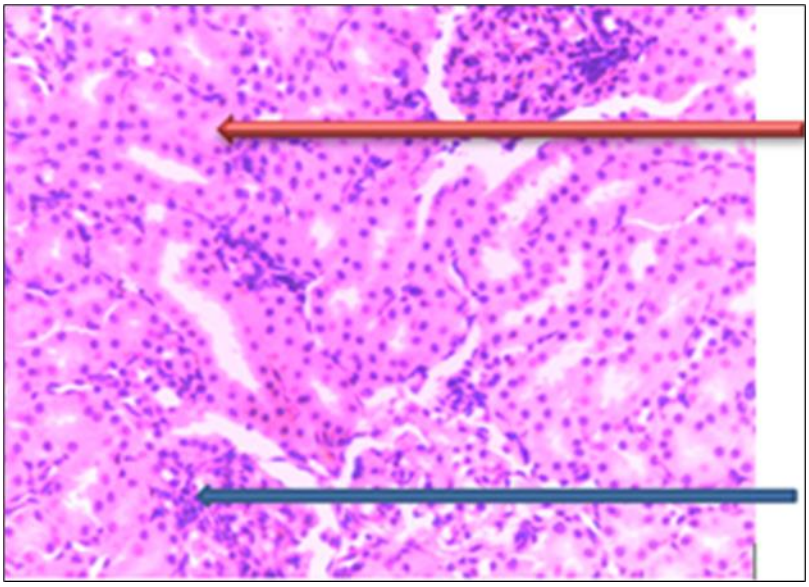


Figure 6 Section of kidney from Group 4 showing marked mesangial proliferation and fibrosis (blue). The tubules are normal (red) (H&E ×400)

4. Discussion

The effects of Exluton and Combi-3 on the kidney function is as shown above in tables 1&2 for urea, creatinine and serum electrolytes respectively in female wistar rats. The traditional markers of nephrotoxicity and renal dysfunction are blood urea and serum Creatinine which are specific with low sensitivity in the detection of earlier renal damage [16].

From the results as presented in the tables above, there was notable significant elevation in the levels of Creatinine in all doses administered in comparison to the control group at 95% confidence interval and therefore suggests increase in muscle metabolism; this is sequel to the fact that creatinine is produced and excreted at a constant rate which is proportional to the body muscle mass [17]. The significant elevation in the creatinine levels also suggests kidney impairment and this is in consonance] with literature review [18,19]. However, this contradicts the findings [20] which reported no significantly change in mean serum creatinine following 6 cycles of COMBI-3 ingestion containing Drospirenone. Therefore, the measurement of plasma alone should not be used to assess renal function as Plasma creatinine levels may not be affected until significant renal damage has occurred [4].

A significant increase in the Urea level in all doses administered in comparison to the control level at 0.05 p- value was observed and this suggests that oral contraceptives increase plasma urea concentration [21]. Urea levels increase in conditions where renal clearance decreases (in acute and chronic renal failure/impairment) [22] .

The increased variation in the levels of Sodium and Potassium in all doses administered in this study as seen in Table 2 above indicates hypernatremia for increased Sodium level which can be caused by Diarrhea, kidney dysfunction and diuretics [23] and this is in agreement with the studies [24] which suggested that higher than normal levels of Sodium in the plasma is an indication of electrolyte disorder. Increased Potassium level is known as Hyperkalemia, a condition caused by renal impairment associated with high serum potassium level. Chloride and HCO3- levels decreased significantly in the present study except in the groups administered with low dose of Exluton at 95% confidence interval as seen in table 2 in comparison to the control groups. The notable decrease in these electrolytes may be associated with electrolytes disorder as well as renal impairment and this is in agreement with the research carried out [25] and concluded that low serum HCO3- is associated with the risk of chronic kidney disease (CKD); Chloride depletion which is known as Hypochloremia can result from both extrarenal and renal causes [26].

5. Conclusion

The findings of this study opines that both Exluton and Combination-3 may alter the functionality of the kidney, hence its impairment. However, the adverse effects were more in the groups administered Combination-3. Therefore,

occasionally examination on the status of the Kidney is recommended for those on oral contraceptives, so as to evaluate the renal functionality and early detection of possible dysfunctions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare no conflict of interest.

Statement of ethical approval

All animals were handled in accordance with the guide for the care and use of laboratory animals prepared by the national academy of sciences and published by the national institute of health guide for the use of laboratory animals [27].

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