Effects of Oral Contraceptive on the Histology and Biochemistry of the Liver and Kidney of Adult Female Albino Rat

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ABSTRACT

Oral contraceptives (OCs) are the most mainstream sort of conception prevention. The pills stop ovulation and keep the ovaries from delivering eggs. The study aims to evaluate the effect of oral contraceptive on the histology and biochemistry of the Liver and Kidney using female albino rat. Twelve animals were grouped into three groups of four animals (n=3). Group I served as a control; Group II were administered oral contraceptives for seven (7) days while Group III received oral contraceptive for the period of fourteen (14) days. The animals were treated for twenty-one days (21) days, at the end of which they were anaesthetized under Chloroform, sacrificed and their blood serum was collected for biochemical assays. The Livers and Kidneys were collected for histological studies. The result showed the variation in selected Kidney function parameters of rat treated with 0.75mg of Postinor-2, a synthetic hormone. There was a significant increase ($p<0.05$) in Urea and Creatinine in the group served with OCs. Postinor-2 was observed to have a significant time-dependent impact on K, Cl and Na levels. Animals in group 2 experienced no significant increase or decreased ($p>0.05$) in the level of ALP, ALT, AST and Bilirubin but those in group 3 were significant ($p<0.05$) when compared with the control group. Histological sections reveal distorted tubules and vessels. Frequent use of OCs could cause deregulation of the extracellular fluid level, hypertensive disorder, renal and hepatic impairment.

Keywords: Oral Contraceptive, Histology, Biochemistry, Liver, Kidney, Albino Rat

INTRODUCTION

Oral contraceptives (OCs) are the most mainstream sort of conception prevention. The pills stop ovulation, keeping the ovaries from delivering eggs. They additionally thicken cervical fluid, making it harder for sperm to enter the uterus.[1] Oral conceptive may expand a lady's danger to Liver illnesses, Kidney infections, Cerebrovascular sickness and Cervical malignant growth.[2] A woman taking the pills is 1.9 times liable to die from Cerebrovascular related illnesses and 2.5 times liable to die from Cervical disease.[2] Oral contraceptives are taken by about 100 million women around the world. Studies have demonstrated that engineered chemicals utilized for oral preventatives enormously increased the danger of blood coagulation and structures in the legs and can cause injury and death if they travel to the Heart, Lungs and Brain.[3]

Leptin's consequences for body weight are interceded through impacts on hypothalamic foci that control and conduct, internal heat level and energy use. It is a protein chemical with significant impacts in directing body weight, digestion and regenerative capacity. Additionally, leptin can serve as a hunger suppressant. It lessens an excessive amount of the body fat and is more dynamic to burn off more energy. The measure of Leptin found in individual's increases as their muscle versus fat ratio increases.[4]

Combined oral contraceptives (COCs) is the most endorsed contraceptive strategy and is utilized by more than 100 million women around the world.[5] These pills contain an Estrogen part (Ethinyloestradiol, Mestranol, Oestradiol or its derivative Oestradiol valerate) and a Progestrogen (Levonorgestrel, Norethisterone, Gestodene,

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Desogestreldrospirenone, Nomegestrol, Dienogest or Cyproterone).[6] Postinor-2 is an oral preventative tablet containing the synthetic Progestogen (Levonorgestrel). The fast advancement of this preventative strategy, especially as for the decrease in the portion of Estrogen and the union of the new Progestogens has been reported.[7] As of late, new prophylactic regimens, explicitly those including consistent or extended use points toward limiting the chemical-free span between one bundle of pills and another.[8] The consolidated oral prophylactic pill is a compelling strategy that can likewise offer different advantages. Women quickly embraced the pills as they permit the solid partition of sex and multiplication and allowed them the chance to arrange for when to have kids. From that point forward the pill had evolved to guarantee great viability while limiting unfriendly impacts.[6] Women who do not use COCs have essentially lower paces of death from malignancy, Cardiovascular infection and different sicknesses.[8]

Lately, investigations have indicated that oral prophylactic pills may affect Liver and kidney functions. Excess of this medication has been demonstrated to upset ordinary Liver and Kidney morphology. As indicated by Fakhir *et al*., (2016),[9] oral preventative pills influence the biochemical boundaries of the Kidney, with raised degrees of Creatinine, Urea and Electrolytes. Oral preventative likewise affects Liver capacity. Raised degrees of Acid phosphatase, Aspartate aminotransferase and Alanine aminotransferase have been found in people on oral contraceptives.[9]

Oral Contraceptive is a medication ordinarily utilized for the prevention of pregnancy, over 200 million women overall accept oral preventative pill as methods for forestalling origination.[10] Oral preventatives are predominantly manufactured chemicals that disturb the normal hormonal cycle in a woman which may cause a lot of issues ranging from Cardiovascular illness to Kidney and Liver sicknesses. Many women because of easy access have assumed control over dosing of this medication, for the most part, because of the non-limitation and accessibility of the medication in various patent medication stores. Consequently, they may be causing destruction of their Kidney and Liver since these organs play significant roles in the metabolism and discharge of medication metabolites.[10]

Therefore, the purpose of this study is to evaluate the histological and biochemical effect of oral contraceptive on the Liver and Kidney. Information obtained from this study will be of importance in patients' guidance and counselling.

MATERIALS AND METHODS

Procurement of Oral Contraceptive

The oral contraceptive (Postinor-2) product name (0.75mg Levonorgestrel) Generic name used for the study was purchased at Ampuh Toa Pharmacy 109 Chime Avenue, New Heaven, Enugu.

Animal Housing

Twelve (24) adult albino rats of both sexes weighing between 100-145g were obtained from the animal house facility of the Veterinary medicine, University of Nigeria Nsukka, Enugu State. The animals were housed in steel wire cages and allowed to acclimatize for one week under standard conditions of temperature 25°c ± 3°c). The rats were fed with standard pellets (Guinea feed Nigeria®, PLC) and water ad libitum. All animals in this study were handled according to international guidelines for handling experimental animals.[11]

Ethical consideration

The study ethical approval was granted by College of Medicine Ethical Committee (COMREC), University of Nigeria with an approval number: 076/08/2018. The study was also conducted in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animal.[11]

Experimental Design and Conduct

Animals were grouped into three groups of four animals (n=4). Group I animals served as vehicle control, Group II animals were administered oral contraceptives for the period of 7 days, while

Group III animals were administered oral contraceptive for the period of 14 days. All drugs were administered intraperitoneally. The animals were kept for twenty-one (21) days, at the end of which they were anaesthetized under Chloroform, sacrificed and their blood was collected for biochemical assays and the Liver and Kidney were fixed in 10% formalin for histological studies.

Biochemical Tests

At the end of the 21st day, the animals were weighed, blood samples were collected by retro-orbital puncture from the medial canthus of the rats, the serum was separated from each blood sample and some biochemical parameters were evaluated.[12]

Serum Alkaline Phosphatase (ALP) estimation was by the enzymatic method of Reitmen and Frankel (1957).[12] Serum Electrolytes were estimated using a machine and method designed by Champion, Pellet and Grenier called Flame Emission Spectrophotometer Method. Sodium and potassium concentration were determined using the Flame Emission Spectrophotometer, while Chlorides concentration was determined using same.[13]

Relative Organ Weight

The rats were sacrificed under Chloroform anesthesia and dissected. The Liver and Kidney of each rat weighted to determine the relative organ weight (ROW) of each organ which is calculated thus:

ROW = Absolute organ weight (g)/bodyweight of rat on sacrifice (g) x 100/1

Histological Processing

The excised Liver and Kidney were cut in slabs of about 0.5µm thick and fixed in 10% formalin. The tissues were processed with paraffin wax embedding medium using an automatic tissue processor.[14] Hematoxylin and Eosin staining was done according to Omorodion *et al.,* 2018.[14]

Microscopy and Photomicrography

Tissue sections were examined using a microscope designed by Takeshi Yamashitanamed Olympus binocular microscope with in-built lighting system. The sections were photomicrographed using a digital microscope camera (Samsung Model SS & 50) attached to an Olympus trinocular microscope.

Statistical Analysis

Results were expressed where appropriate as mean ± standard deviation (SD). Differences between valves were determined with one-way analysis of variance (ANOVA) $P < 0.05$ was considered significant.

RESULTS

Biochemical effect of oral contraceptive on serum electrolyte

The result shows the variations in selected Kidney function parameters of rats treated with 0.75mg of postinor-2 synthetic hormone. The oral contraceptive was observed to be significantly increased ($P < 0.05$) Urea and Creatinine in a matter that is dependent on period of ingestions compared to the control group Furthermore on electrolytes with Kidney Function changes as indicated by K, Cl, and Na. Postinor 2 was observed to have a specifically impact on K level, which was time dependent decrease compared to the control. Although Na was found to decrease but the result was not statistically significant.

Biochemical effect of oral contraceptive on serum ALP

The table below shows the effect of various doses of oral contraceptives on the level of ALP of normal rats. Animals in the control group have a normal concentration of ALP. Animals in group 2 experienced a slight decrease in the level of ALP but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment groups 3 showed significant ($P < 0.05$) decrease in ALP concentration compared with the animals in the control group. Rats administered oral contraceptive for (7 days) showed a non-significant ($P > 0.05$) decrease in ALP while those rats administered with the drugs for 14 days showed a significant decrease in ALP level.

Biochemical effects of oral contraceptives on serum ALT

The table shows the effect of various doses of oral contraceptives on the level of serum Alanine aminotransferase (ALT) of normal albino rats. Animals in the control group have a normal concentration of ALT. Animals in group 2 experienced a slight increase in the level of ALT but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment group III showed significant ($P < 0.05$) increase in ALT concentration compared with the animals in the control group.

Biochemical effect of oral contraceptives on serum AST

The table shows the effect of various doses of oral contraceptives on the level of serum Aspartate aminotransferase (AST) of normal albino rats. Animals in the control group have a normal concentration of AST.

Animals in group 2 experienced a slight increase in the level of AST but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment group III showed significant ($P < 0.05$) increase in AST concentration compared with the animals in the control group.

Biochemical effect of oral contraceptive on serum total Bilirubin concentration

The table 2 shows the effect of various doses of oral contraceptives on the level serum total Bilirubin of normal albino rats. Animals in the control group have a normal concentration of total Bilirubin concentration. Animal in group II experienced a slight increase in the level of total Bilirubin but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment groups 3 showed significant ($P < 0.05$) increase in total Bilirubin concentration compared with the animals in the control group.

Histological Effect of Oral Contraceptive on the Kidney of Adult Female Albino Rat

The result showed normal histology of the treated animals in comparison with the control group, as shown in the figures below. But there was a slight alteration that was not necessarily significant in the Kidney cells in group 3. The result may reflect the safety of Postinor pills, as previous studies referred to the wide use and safety of this drug. Despite the presence of biochemical changes related to the Kidney.

Table 1: Shows the biochemical effect of oral contraceptives on the Kidney of an albino rat on oral conceptive at different time intervals and was expressed in mean plus standard.

Kidney Function Parameters	Group 1 (Control)	Group 2 (OCs for 7days)	Group 3 (OCs for 14days)	*p*-value
Urea Mmol/L	6.46 + 2.040	25 + 0.05	19.76 + 1.97	$p < 0.05$
Creatinine Mmol/L	152.93 + 22.10	5746.11 + 414.60	260.79 + 29.17	$p < 0.05$

The values obtained in the above table are statistically significant at $p < 0.05$.

Table 1 shows the biochemical effect of oral contraceptives on the Kidney of adult female albino rat on oral conceptive at different time intervals. Group 1 served as the control, group 2 was administered the oral contraceptive for 7 days while Group 3 were administered the oral contraceptive for 14 days.

Table 2: Shows the variations in selected Kidney function parameters of rats treated with 0.75mg of Postinor-2 synthetic hormone.

Electrolyte	Group 1 (Control)	Group 2 (OC's for 7days)	*p*- value for 14days)	Group 3 (OC's	*p*-value
K (Mmol/L)	4.05+0.92	8.63+1.90*	0.03	13.58±1.86*	0.02
Cl (Mmol/L)	101.00±2.83	92.33±2.08	0.06	83.75±5.66	
Na (Mmol/L)	136.00±1.41	111.67±10.41	0.0 9	121.25±3.77	

Investigation on the effects of oral contraceptives on the Liver and Kidney organs of adult female albino rats were carried out. The biochemical effects were first evaluated in this work: Biochemical analysis and *histological* examination of the Liver and Kidney organs. Results obtained for the parameters assessed are shown below.

Table 3: shows the biochemical effect of oral contraceptives on the Liver of an albino rat on oral conceptive at different time intervals and was expressed in mean plus standard deviation.

LFT	Group 1 (Control)	Group 2 (OC's for 7days)	*p*-value	Group 3 (OC's for 14days)	*p*-value
ALP (n/l)	84 ± 2.96	35 ± 14.20	0.08	14+ 2.51*	0.001
ALT (/i/Z)	22 ± 3.25	25.00 + 6.69	0.79	50 ± 0.33*	0.009
AST (/r/Z)	96 ± 99	99± 1.23	0.99	118 ±2.78*	0.023
TB (mg/dl)	0.91± 0.22	1.1± 1.10	0.08	1.8 ±2.22*	0.021

The sign (*) denote significant at value less than or equal to 0.05

Keys: TB (total bilirubin), CB (Conjugated bilirubin), AST (Aspartate Transaminase), ALT (Alanine transaminase), ALP (Alkaline phosphatase.

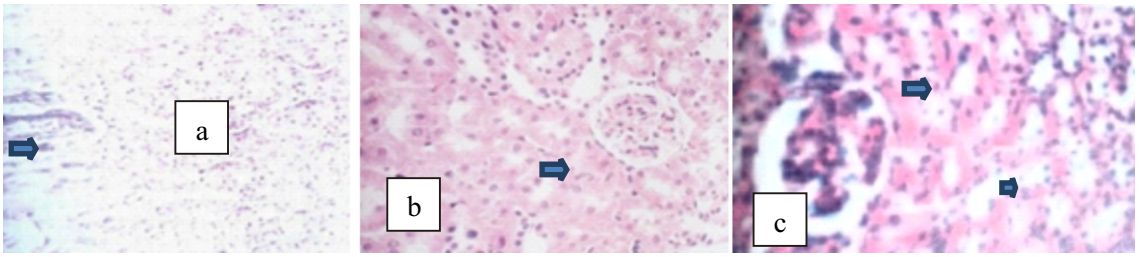

Figure 1: Photomicrograph (a)showing a section of Kidney with normal convulated tubules, urinary space, renal corpuscle and glomeruli, Photomicrograph (b) showing normal section of Kidney treated for 7 days with oral contraceptive, Photomicrograph c, showing section of Kidney treated for 14 days with disrupted tubules and vessels (arrows).

Histological effect of oral contraceptive on the Liver

The result showed normal histology treated animals in comparison with the control group, as shown in the figure below. But there was a slight alteration that was not necessarily significant in the Liver cells in Group III.

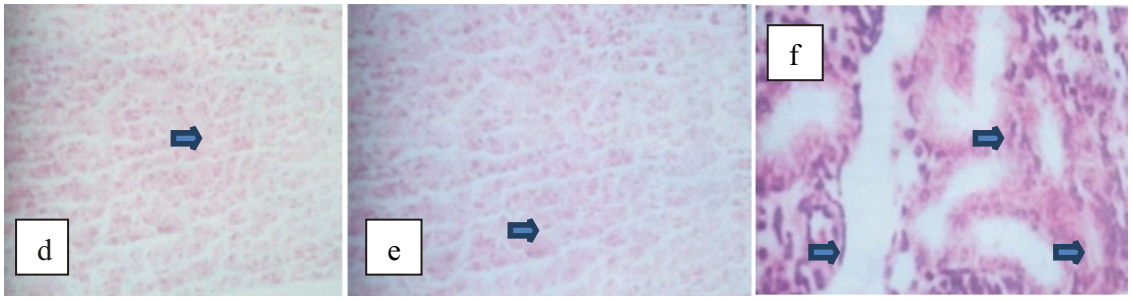

Plate 2: Photomicrograph of normal liver tissue section representing the control (d), photomicrograph of liver tissue (e)treated for 7 days showing normal histological architectures, Photomicrograph (f) treated for 14 days showing diffused vacuolation as well as periportal inflammation. There is lymphoid aggregation at the focal area, the nuclei of the hepatocyte appear hyperchromatic. Stain H&E. MAG X400.

DISCUSSION

The two most influential female sex hormones include Oestrogen and Progesterone, change in concentration across the menstrual cycle and are caused by oral contraceptive usage. In the present investigation, it was observed that oral contraceptive containing Levonorgestrel significantly increased the Creatinine output suggesting an 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. The mean significant increase is in line with the study of Oelker *et al*., (2005),[15] who studied oral contraceptive containing antimineralocorticoid Progesterone, but his research contradicts the study of Taneepanichskul *et al*., (2007),[16] who reported no significant change in the mean value of Creatinine following 6 cycles of oral contraceptive ingestion. Although depressed levels of Creatinine are rare and are not clinically significant, its excretion is an indication of renal impairment and are regarded as the most important marker for the diagnosis and treatment of Kidney disease and measured primarily to ascertain Kidney diseases.

From the research, findings on electrolyte with Kidney function showed that oral contraceptive containing 0.75ml of Levonorgestrel significantly increased plasma Na+ and K+ but decreased plasma Cl. This study is following several other studies Oeker *et al*., (2005).[15] The results from this study suggest that oral contraceptive usage may alter the fluid nature of extracellular fluid.

Thus, understanding the interaction between oral contraceptive and fluid regulatory system is crucial. Female sex hormone has been reported to influence Sodium and water distribution and thus, fluid compartment volumes and dynamics and may not be unrelated to the hypersensitive effect of oral contraceptive usage.[15] This mechanism behind the effect of oral contraceptive used in this study may be explained by the fluid retention potentials by activating the renin angiotensin aldosterone system, enhances vasodilation, capillary permeability and lower the operating setpoint of plasma osmolality by Oestrogen.[15] Progesterone has been known to counter the effect of Oestrogen by competing with mineralocorticoid receptor as Aldosterone which may cause natriuresis.

From the study, it can be deduced that prolonged use of oral contraceptive affects Liver function parameters. There was no significant increase in the level of serum ALT, Bilirubin and AST in the animal group treated for 7day, but there was a significant increase in the level of serum ALT, Bilirubin and AST.

In the animal group treated for 14 days, there was a marked increase in the lever of AST. This might be as a result of the oral contraceptive on the Heart. AST is also a marker of cardiac muscle damage. There was no significant decrease in the level of ALP in the animal group treated for 7days as compared with the level of ALP in the animal group treated with an oral contraceptive for 14 days.[15]

The animal group treated with 0.75mg of Levonorgestrel for 7days did not experience any form of kidney or Liver damage. This might be suggestive of the safety of the drug for women's consumption. The animal group that was treated with the drug for 14 days experienced slight alteration in the morphology of the Liver and Kidney but the result was not significant as compared with the control group.

CONCLUSION

Frequent use of oral contraceptive could cause deregulation of the extracellular fluid, can lead to hypertensive disorder and renal impairment. Also, frequent use of oral contraceptive could lead to Liver impairment.

RECOMMENDATION

More research should be carried out on the effect of oral contraceptive on the heart. More awareness sessions on health for mothers who are going on oral contraceptive should be implemented in school, audio-vision systems, and local organization. Deeper and screening research studies about

the effect of oral contraceptives intake on some other anabolic steroid hormones such as those of the thyroid could be addressed and conducted with a higher level of funding the from Ministry of Health [MOH].

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