

*Full Length Research Paper*

## **The "Date Rape" drug abuse: Implications on the female reproductive system**

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Rohypnol is hypnotics that are commonly used to treat insomnia, anxiety and seizure disorders. It has been abused by both sexes and club men often drop the tablets into women's drinks to create a potential rape situation. Thirty-six animals (Wistar rats) were used for this study. They were grouped into three (N=12); Group 1 (Control) received 0.04 ml/kg body weight (bwt) of distilled water, Group 2 (Normal dose) received 0.04 mg/kg bwt of rohypnol and Group 3 (overdose) received 0.08 mg/kg bwt of rohypnol. The exposure was done once every week for three weeks, and three animals per group were sacrificed every 24 h post-administration. Blood samples were collected at every time of the sacrifice for hormonal assessment and the ovaries and uterus were removed and preserved for histological analysis. The last batch of animals was sacrificed 3 weeks after the third exposure. The results obtained from the hormonal analysis showed statistically significant ( $p < 0.05$ ) reduction in estrogen production for all groups 2 and 3 animals at a repeated normal dose (2nd and 3rd exposures) when compared with the control. The group of animals that received the overdose and repeated overdose of rohypnol showed high statistically significant decrease in all the analyzed reproductive hormones (Estrogen, Progesterone, Luteinizing and Follicle-stimulating hormones); while the hormonal pattern of the last batch of animals across all groups, showed a perfect correlation with the control. Histology of the ovary and the uterus, however, showed no pathological changes in all the groups. This work has concluded that repeated uses and the overdose of the drug can disrupt the normal hormonal profile of the female reproductive system which may be corrected naturally on stoppage of exposure.

**Key words:** Rohypnol, hormones, sedatives, overdose, histology.

### **INTRODUCTION**

Globally, about one in ten girls (around 120 million) under the age of 20 have been raped or sexually assaulted

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(UNICEF, 2014; Gilbert et al., 2019). It has been estimated that over a lifetime, one in three women will experience sexual violence, regardless of country, background or age (UNICEF, 2014; Kerr et al., 2003; Martin et al., 2020). Rape is now a global epidemic and has been identified as a part of the continuum of sexual assaults committed against women (Martin et al., 2020; Kavanaugh, 2012). Though the history of rape was as old as human civilization itself (Cyril, 1974), the reports of drug-facilitated rapes started receiving public attention in the early 90's. In 1993, rohypnol facilitated rape incidences were recorded in the United States of America (Calhoun et al., 1996; Doheny, 1996; Bullock, 1996; Singh et al., 2014). One in every six college women in Canada has reported experience of drugged rape assault (Girard and Senn, 2008). Recent study also revealed that over 11 million women have been raped while drunk or drugged in the United States (Sandal, 2020).

Rohypnol was a round white tablet produced in 1 or 2 mg. They are tasteless, odourless, and quickly dissolve in liquid (Sandal, 2020). The manufacturer reformulated the drug after it was implicated in drug-facilitated rapes; the new products now appear as green tablets, oblong with inclusion of a dye that turns blue when dissolved in liquid. This was done to make the drug more easily detectable especially in some colourless drinks (Gautama et al., 2014). Previous literature has shown that there is an increase in the number of claims of Drug facilitated assaults, which could be due to the advent of drugs that can be easily administered without the victim's knowledge (Hall et al., 2008; Sandal, 2020).

Since 1993, researchers have conducted various studies on the abuse of this drug (Anderson et al. 2017). Its legal and ethical implications have also been thoroughly publicized; the inability of the victims to recollect the situation surrounding the assaults nor able to recognize assailants, makes multiple exposures quite possible (Vagianos, 2017). This study therefore, documented the effects of overdose and multiple exposures to rohypnol on the female reproductive system, by assessing the hormonal functions and the organs histomorphology.

## MATERIALS AND METHODS

### Procurement of rohypnol

Four milligrams of rohypnol were purchased at Binji Pharmacy, Sokoto after obtaining a prescription from Gynaecology Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

### Experimental animals

Thirty-six adult, female Wistar rats with an average weight of 300 g were procured from the animal holdings of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. They

were kept in metallic cages in an air-conditioned environment with three rats per cage and maintained at room temperature of (25 ± 2°C) under 12 h dark and light cycle. The animals have free access to feed grains and water and were acclimatized at this condition for two weeks at the animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, where the study took place. Before the commencement of the study, physical examinations and vagina smear analysis of the animals were carried out. They were found to be in a very good state of health without pregnancy.

All experimental protocols were done in compliance with the Usmanu Danfodiyo University Ethical Committee on the Use of Animals for Research (ECUAR), International Animal Care and Use Committee (IACUC) in Nigeria as well as 1985 National Institute of Health accepted guidelines for laboratory animal use and care.

### Animal grouping and drug administration

The thirty-six Wistar rats were grouped into three (3), designated as: Group 1 (Control [C]), Group 2 (Normal dose [N]), and Group 3 (Overdose [O]). Each group consists of 12 rats which were further sub-divided into 4 groups with 3 rats each: Control (C1, C2, C3 and C4); Normal Dose (N1, N2, N3 and N4); Overdose (O1, O2, O3 and O4) to mimic the manner of the human drug intake.

Group 1 (Control group) received 0.03 ml/kg body weight (bwt) of Distilled water, Group 2 (Normal dose group) received 0.04 mg/kg bwt of rohypnol and Group 3 (overdose group) received 0.08 mg/kg bwt of rohypnol (de Almeida, 2010). The drug administration was done in a single dose (not exceeded 1 ml/100 g bwt) by gavage using intubation cannula, once every week for three weeks to evaluate the effect of single and multiple exposures to the normal and overdose of the drug.

### The technique for collection of blood samples

Blood was collected from chloroform anaesthetized rats by cardiac puncture into plain bottles, centrifuged at 3000 revolutions/min (3000 rpm) for 10 min (Kumar et al., 2017). The serum of each sample was separated into cryovial and stored at -20°C until required for analysis.

### Format for the sacrifices

Three animals per group were sacrificed by cervical dislocation at every 24 h post-administration, as illustrated here on weekly basis: Week 1- C1, N1, O1; Week 2- C2, N2, O2; Week 3- C3, N3, O3 and Week 6- C4, N4, O4. The last batch of animals (C4, N4, O4) was sacrificed 3 weeks after the third administration.

### Hormonal assessment

The quantitative determination of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentration in the rats' serum was done by immune-enzymometric assay (IEMA/ELISA) according to the manufacturer's instructions, while estradiol (E) and progesterone (P) were measured by radioimmunoassay (Pantex kit, Santa Monica, CA) (Burowa et al., 2019). The analysis was done at the Department of Chemical Pathology, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

### Histological preparations

The ovaries and uterus of the animals were carefully removed, fixed

**Table 1.** Comparison of the mean  $\pm$  standard deviation of serum LH, FSH, progesterone and estrogen of the rats in group C1 with N1 and O1 Group, and N1 with O1.

Group	LH	FSH	Progesterone	Estrogen
CI	3.62 $\pm$ 0.04	0.96 $\pm$ 0.03	2.68 $\pm$ 1.04	320.60 $\pm$ 0.02
N1	3.76 $\pm$ 0.03	0.82 $\pm$ 0.03	2.07 $\pm$ 0.08	349.60 $\pm$ 2.97
P-value	0.14	0.15	0.10	0.08
O1	4.29 $\pm$ 0.03	0.49 $\pm$ 0.04	1.46 $\pm$ 0.04	712.95 $\pm$ 5.02
P-value	0.03	0.06	0.03	0.01
N1	3.76 $\pm$ 0.03	0.82 $\pm$ 0.03	2.07 $\pm$ 0.78	349.60 $\pm$ 2.97
O1	4.29 $\pm$ 0.03	0.49 $\pm$ 0.04	1.46 $\pm$ 0.04	712.95 $\pm$ 5.02
P-value	0.00	0.01	0.01	0.00

with 10% Formol saline and processed for paraffin embedding. Sections were cut at 3  $\mu$  using Leica Rotary Microtome, stained with Haematoxylin and Eosin stains and examined under light microscope (Orchard and Nation, 2012; Muhammed et al., 2016). The tissue processing and examination were done at the Department of Histopathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

#### Data analysis

The data obtained from the hormonal assessment were analyzed using the statistical package for social science (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were represented as the mean  $\pm$  standard deviation (S.D). Student T-test at 95% confidence interval was used to determine the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when  $p < 0.05$ .

## RESULTS

Reproductive activity in female rats, like in human, is a cyclic event that are largely coordinated and regulated by hormones (Durlinger et al., 2001). Hormones are physiologic chemical substances, synthesized and secreted by ductless endocrine glands (Hafez et al., 2000). Reproductive hormones have been widely accepted as markers of fertility in gynaecology (Hafez et al., 2000) and the study of their production and mechanisms of regulation is a discipline of its own in reproductive health.

Two hormones (FSH and LH) produced by the gonadotropes were selected; one of the anterior pituitary cells and another two hormones (Estrogen and Progesterone) from the groups of gonadal hormones produced by the ovaries (Hafez et al., 2000).

## DISCUSSION

The results of the hormonal analysis are shown in Tables

1 to 4 for all the estimated parameters. There is a dearth of literature on the effects of rohypnol on the female reproductive system and this work is a case-control study, designed mainly for control comparative analysis.

Table 1 shows a comparison of mean from the control animals with the normal and overdose group separately for the first week. There are no statistically significant changes between the control C1 and the normal dose N1 at the first exposure, while changes in the overdose group O1 are significant for all hormones except FSH ( $p$ -value 0.06). Comparison between the normal N1 and overdose O1 is however, highly significant across the groups at the first administration. This could be a pointer to the danger of overdose of rohypnol which those abusing the drug take no cognizance of.

Results of the second exposure took a similar pattern with the first, except that LH was significantly reduced when the second control C2 was compared with the second normal dose N2. Comparison of the means of the second normal group N2 and second overdose O2 showed highly significant values across the groups.

Like in the second exposure, LH, and also estrogen showed highly significant values in the N3 when compared with the control C3. The pattern of significance was however in the opposite direction. While LH production was reduced, estrogen continues to be overproduced across the group. This is an aberration in the reproductive cycle as the production of LH supports the ovarian endocrine cells for estrogen production (Bowen, 2004). All others, C3-O3 and N3-O3 showed very high significant values at the 3rd exposure.

The activities of the reproductive hormones have been widely studied. FSH and LH act synergistically in female reproduction and during the early follicular phase of the oestrous cycle; FSH is the parameter of measurement for fertility. At that time, the levels of estradiol and progesterone are usually at the lowest point of the cycle. The theca cells in the ovaries that provide hormonal precursors for estradiol production are supported by LH (Bowen, 2004). At the time of menstruation, FSH initiates

**Table 2.** Comparison of the mean ± standard deviation of serum LH, FSH, progesterone and estrogen of the rats in group C2 with N2 and O2 group, and N2 with O2.

Group	LH	FSH	Progesterone	Estrogen
C2	3.70±0.03	0.96±0.02	2.63±0.08	320.70±1.98
N2	2.10±0.05	0.85±0.04	2.27±0.15	290.90±3.11
P-value	0.00	0.08	0.09	0.08
O2	3.42±0.03	0.45±0.05	1.28±0.05	714.50±2.40
P-value	0.01	0.01	0.01	0.00
N2	2.10±0.05	0.85±0.04	2.27±0.15	290.90±3.11
O2	3.42±0.03	0.45±0.05	1.28±0.05	714.50±2.40
P-value	0.01	0.00	0.00	0.00

**Table 3.** Comparison of the mean ± standard deviation of serum LH, FSH, Progesterone and Estrogen of the rats in group C3 with N3 and O3 Group, and N3 with O3.

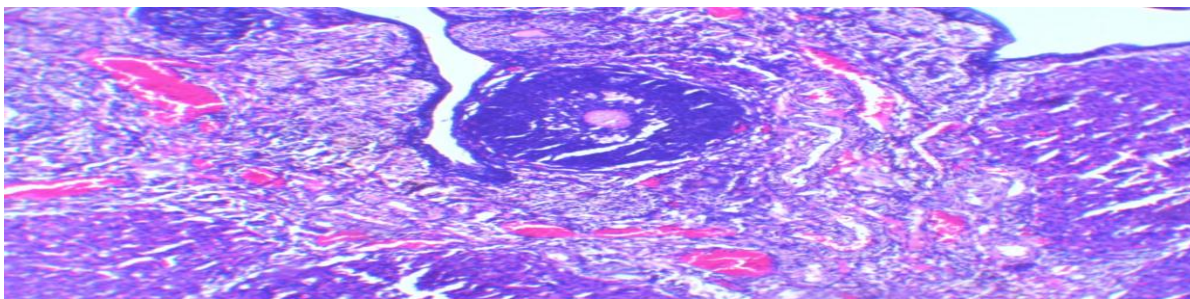
Group	LH	FSH	Progesterone	Estrogen
C3	3.79±0.14	0.92±0.01	2.63±0.03	319.75±2.33
N3	0.83±0.02	0.89±0.03	2.70±0.03	236.45±4.03
P-value	0.00	0.41	0.15	0.00
O3	2.20±0.02	0.32±0.02	1.26±0.05	664.00±1.84
P-value	0.00	0.00	0.00	0.00
N3	0.83±0.02	0.89±0.03	2.70±0.03	236.45±4.03
O3	2.20±0.02	0.32±0.02	1.26±0.05	664.00±1.84
P-value	0.00	0.00	0.00	0.00

**Table 4.** Comparison of the mean ± standard deviation of serum LH, FSH, progesterone and estrogen of the rats in group C4 with N4 and O4 Group, and N4 with O4.

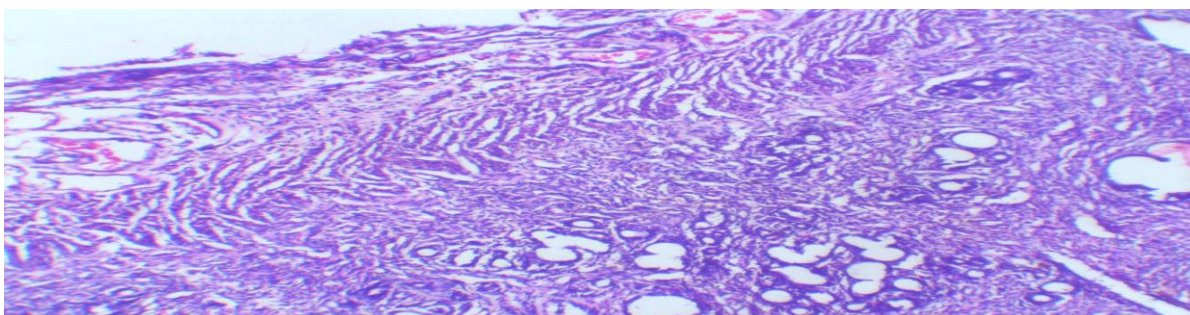
Group	LH	FSH	Progesterone	Estrogen
C4	3.64±0.24	0.93±0.01	2.70±1.00	319.10±0.01
N4	3.68±0.02	0.88±1.03	2.56±0.02	329.10±0.22
P-value	0.15	0.12	0.10	0.20
O4	3.91±0.01	0.69±0.02	2.26±2.07	403.85±0.47
P-value	0.08	0.07	0.17	0.09
N4	3.76±0.03	0.82±0.03	2.07±0.78	349.60±2.97
O4	3.99±0.01	0.79±0.09	2.30±1.01	356.00±3.07
P-value	0.06	0.11	0.08	0.09

follicular growth, specifically affecting granulosa cells (Bowen, 2004). With the rise in estrogens, LH receptors are also expressed on the maturing follicle, which causes

it to produce more estradiol. Estrogens are usually present at significantly higher levels in women of reproductive age. They involved in the thickening of the



**Figure 1.** Photomicrograph of the ovarian tissue showing normochromic and normocytic cell (H&E X400).



**Figure 2.** Photomicrograph of the Uterus tissue showing normal uterine cells (H&E X400).

endometrium and other aspects of regulating the menstrual cycle (Hill et al., 2004). So, any drug that affects the production or action of one is indirectly affecting the other, because of their chain reaction activities.

Results of the fourth group brought with it, a glad tidings, which confirmed the report of natural normalization of the oestrous cycle after the stoppage of rohypnol administration. Three weeks after the last exposure, all parameters estimated in this group (C4-N4, C4-O4 and N4-O4) showed no significant difference across all its sub-groups.

The gross and the histopathological examination of the ovary and uterus tissues (Figures 1 and 2) from both experimental and control rats showed normal structure and absence of any pathological lesion in all the organs.

## Conclusion

This study has established the possible disruption of the oestrous cycle by multiple exposure and/or overdose of rohypnol in the animal model. The pattern of this result can be a replica in human being because of the closely related genetic composition shared with the experimental model used in this study. Contrary to the widely circulated rumour in India (Kapoor, 2006) and Nigeria about the positive correlation of rohypnol exposure to female

infertility; this study has proved otherwise. Though, caution has to be taken, even among women that are using rohypnol on normal medication, because of its effects on the reproductive hormones.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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