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The Noxious Effects Of *Perinatal* Exposure To Different Doses Of Chlorpromazine On Fetal Growth And Development In Albino Rats (*Rattus Norvegicus*).

Jane Kuria[1], Joseph Kweri[2], Caroline Sigei[3], Walter Rono[4], James Mwangi[5], Ann Nyaga[6],joseph wachira,[7] Jennifer Segut[8]

[1-6](*Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES) Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.*

ABSTRACT:
The in-utero effect of Chlorpromazine on fetal growth and development has not gotten explained. Thus, this study purposed to evaluate the prenatal effects of this compound on fetal growth and development in albino rats, including the mean fetal weight, the length of the Crown lump, the diameter of the bi-parietal, and the head circumference when applied to albino rats at various doses and gestational periods. The experimental post-test design was used. Thirty albino rat dams were used for the experiment. To evaluate the teratogenic effect of chlorpromazine when exposed in utero. The 30 albino rat dams were split into two major study categories, consisting of 3 control rats and 27 experimental rats. To determine whether the teratogenic implications of chlorpromazine are dose-dependent, the 27 rats were separated into three sub-groups of 9 rats, each based on the dosage: low, medium, and high. The nine rats in the aforementioned experimental groups were subsequently separated into three groups based on the times of exposure (TM1), (TM2), and (TM3) to determine whether the teratogenic effects of chlorpromazine are time-dependent. Standard rodent pellets and water ad-libitum were provided for all the rats. All rats were humanely sacrificed on the gestation day 20th. The fetal growth and developmental indicators were recorded and evaluated for inferential statistics using SPSS version 25. The outcomes statistical significance was determined using a turkey post hoc multiple comparison test, and all values whose $p<0.05$ got considered important. This study illustrated that chlorpromazine is a fetal growth enhancer at low doses and growth inhibiter at high doses when administered in all trimesters compared with the control. However, effects on prenatal development and growth are dose-dependent but not time-dependent. More research on non-human primates is advised to confirm its safety.

Keywords*: in-utero effects, chlorpromazine, teratogenic effects.*

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I. INTRODUCTION

Chlorpromazine, a first-generation antipsychotic medicine derived from the dimethyl-phenothiazine class, has a chemical formula and a C17H19C1N2S molecular weight of 318.9 g/mol. (Iqbal et al., 2005). This medicine is widely prescribed in the management of maternal psychosis in resource-limited countries of Africa, and Kenya included owing to its cost-effectiveness, efficacy, and easy accessibility (Marangu *et al.*, 2021). However, its noxious effects on fetal growth and development when exposed prenatally have been a subject of controversies; where some studies have reported that it causes severe fetal limb reduction defects, while some other studies have reported that children born of mothers who used chlorpromazine were taller than general population regardless of whether or not they were breastfed (Patton *et al*., 2002). Though all antipsychotic medicines are classified in the class C medicines by the American Food and Drugs Administration(FDA)(Ahmed et al., 2016) and therefore they should not be used during pregnancy, Chlorpromazine has at times been recommended in the management of maternal psychosis when its therapeutic benefits to the mother outweigh the pregnancy risks(Emeka, 2015)(Goldstein et al., 2000). This study aims to assess the harmful effects of perinatal exposure to chlorpromazine at various doses and gestational stages on fetal growth and development markers. This is to generate a repository of scientific data that would form a future bench-marking platform that would guide future scientists and clinicians in determining the safety indices in prescribing chlorpromazine to expectant mothers during pregnancy.

II. Material and method

Study area: This research was carried out at (JKUAT), School of Biomedical Science in the Small Animal Facility for Research and Innovation (SAFARI).

Study Design: In order to conduct this investigation, a post-test-only control experimental study design got used because it's simple to forecast the outcomes.

The Study animals: As the experimental animal model, 30 pure-bred, nulliparous albino rats weighing between 200 and 250g at 8 to 9 weeks were used. The SAFARI Animal House of JKUAT provided these 30 rats from a pure colony. This breed of Albino rats was chosen as the experimental model because of the following known scientific facts as per the study (Anatomy, 2020) (i) they are cheap to maintain, (ii) they have a short gestational period of 21days, (iii) they have a big litter size between 11 and 16 fetuses, and finally (iv) the frequency of spontaneous congenital abnormalities is minimal.

Acquisition of the rats: These 30 albino rats were obtained from (JKUAT) (SAFARI).

Acclimatization of the rats: Before the research could begin, all of the rats were housed in cages at SAFARI Animal House for a week to get used to the experiment.

Sample size determination:

The resource equation for the one-way analysis of variances was adopted in determining the number of rat dams that were to be used in the study. This equation was adopted because it can be applied in animal experiments(Charan & Kantharia, 2013) and based on the existing literature from previous studies, where the standard deviation and the effect on size were unavailable (Arifin & Zahiruddin, 2017). The degree of freedom in the analysis of variance (ANOVA) was based on a predetermined sample size, and the value "E" was measured and used as such in the procedure. The formula is n = DF/k + 1, where DF = the total number of subjects, k = the number of groups, and n = the number of subjects per group (Charan & Biswas, 2013). Therefore, n=20/10+1 =3. The sample size of the fetuses was determined using the convenient random sampling method. From each pregnant female albino rat in the study, three fetuses were selected; thus, the total number of fetuses for analysis was 3x30= 90 fetuses.

Grouping of rats into various study categories: The 30 albino rats were grouped into two large study groups consisting of three control rats and twenty-seven experimental rats to determine whether chlorpromazine has any negative impacts on embryonic growth and development. The Second level was to evaluate whether the observed noxious effects of chlorpromazine are dependent on the doses of exposure. To do this evaluation, the 27 experimental rats were subsequently separated into three subgroups of 9 rats according to the dosages of chlorpromazine used: low, medium, and high. The first batch of 9 rats were for low chlorpromazine doses that was administered at 3Mg/Kg Bw; the second batch of 9 rats was for the medium chlorpromazine dose of 10 Mg/kg/Bw, while the third batch of 9 rats was for the high Chlorpromazine dose of 30mg/Kg/Bw. The third level was to evaluate whether the observed deleterious effects of *in-utero* exposure to chlorpromazine depend on the exposure's gestation periods. To conduct this evaluation, the nine rats in each of the three dose groups described in level two above were further divided into three subgroups, each consisting of three rats, based on the trimesters during which they were exposed to chlorpromazine. The first batches of three rats in each category were exposed to their respective Chlorpromazine doses at trimester one TM1 [i.e., from Gestational. The third batch of three rats in the three-dose categories—Low, Medium, and High—were exposed to their respective chlorpromazine doses at trimester three (TM3), from gestational day 14 to gestational day 20. The second batch of three rats in the three-dose categories—Low, Medium, and High—were exposed to their respective chlorpromazine doses in trimester two, from gestational day 7 to gestational day 20.

Mating of the animals and determination of pregnancy:

Mating was done after a week of acclimatization. One mature male albino rat from the same pure colony as the dams' breeding colony was placed into the cage containing two female albino rats overnight to achieve this. This was to allow enough time to ensure mating took place. The males were then put into their separate cages the following day. Mating was confirmed by obtaining vaginal smears from the mated females and examining them for the presence of spermatozoon. In contrast, pregnancy was confirmed by finding the presence of polyhedral epithelial cells on the vaginal swabs microscopically.

Feeding the rats: All the albino rats in both the control and experimental groups were provided with standard rodent pellets acquired from Unga feeds Limited-Kenya and water *ad-libitum*.

Determination of the chlorpromazine doses, reconstitution, and administration:

The dosages of chlorpromazine used were as follows; a minimum dose of 3mg/kg BW, a medium dose of 10 mg/kg BW, and 30 mg as the high dose adopted by Zahra and colleagues(Zamani et al., 2015). On the other hand, conventional chlorpromazine tablets are sold in formulations of 25mg, 50 mg, and 100 mg. These Chlorpromazine tablets of 100 mg from Cosmos batches 211435 And date of manufacture being Sep. 2020 with an expiry date of Aug 2023, were obtained from the registered pharmacy in Nairobi County. The medicines were reconstituted using distilled water obtained from PAUSTI laboratory JKUAT. A simple guide on converting human dosage to animal dosage was applied, as per Nair Jacob (2016).

Drug administration: All TM_1 animals: - (LCPMG 3mg/kg, MCPZG 10 mg/kg, HCPZG 30 mg/kg) categories received CPZ from GD1-GD20. All TM_2 animals: - (LCPMG 3mg/kg, MCPZG 10mg, HCPZG 30 mg/kg) - categories received CPZ doses from GD7-GD20. All TM_3 animals: - (LCPMG 3mg/kg, MCPZG 10mg, HCPZG 30mg/kg) categories received CPZ doses from GD14-GD20.

Sacrificing the animals: All animals were sacrificed humanely on day 20 using concentrated carbon monoxide, and viable fetuses were harvested (Rai & Kaushik, 2018).

Ethical consideration: all animal procedures were carried out per the protocols by the international Animal research institute (IARI) and the care of the laboratory animal's guidelines with approval from the Animal Ethics Committee-University of Nairobi (Ref: FVM BAUEC/2021/326.

Statistical analysis: The information was gathered using a structured checklist, entered into a computer using an excel spreadsheet for Windows version 10, and then exported to the Statistical Package for the Social Scientist (SPSS) version 25 for analysis. The information included fetal weight, crown lump length, head circumference, and bi-parietal diameter. Turkey's post hoc multiple comparison tests were used to establish statistical significance to determine the effects. The results were deemed significant whenever the value was less than 0.05 ($p<0.05$).

III. RESULTS

It was noted that different parameters showed variance in the experimental group against the Control group. The mean fetal weight of the high-dose chlorpromazine group depicted inverse relation with the time of exposure compared to the control group. A statistical significance difference ($P<0.005$) was observed when the drug was given in all trimesters (TM1, TM2, TM3). However, there was no statistical weight difference ($p<0.005$) for LDCPZG and MCPZG across all trimesters in comparison to the control group. Compared to the control group, the effect was more pronounced in animals who received the drug in trimester one as measured by the mean head circumference and mean bi-parietal diameter, which showed an inverse dose-time relationship for both MCPZG and HCPZG. The crown lump length for the LCPZG depicted direct dose relation with the time of exposure compared to the control group.

Table1.1

The intra and inter-group comparative means of fetal weight, crown lump length and bi-parietal diameter of LDCPZ, MDCPZ and the HDCPZ in (TM1, TM2, and TM3) against the control (C).

Study groups	Time of exposure	Mean fetal wt.	Mean CRL	Mean BPD	Mean Head circumference
Control		6.6333±0.2404	4.3667±0.1202	0.8333±0.0333	3.3667±0.1202
LDCPZG	TM1	7.4000±0.4041	4.8000±0.1732	1.2000±0.1732	3.8000±0.1732
	TM2	7.5333±0.3712	4.8000±0.1155	1.2333±0.1764	3.8333±0.1453
	TM3	7.2333±0.1764	4.7000±0.0578	1.0000±0.0716	3.7000±0.0577
MDCPZG	TM1	5.6000±0.3055	3.9000±0.5774	0.6000±0.1155	2.9333±0.0667
	TM2	5.8000±0.2000	4.1000±0.5774	0.7000±0.0577	3.1000±0.0577
	TM3	5.7333±0.3712	4.0667±0.1333	0.6333±0.0882	3.0667±0.1333
HDCPZG	TM1	3.2000±0.1155*	2.9000±0.5774*	0.3333±0.0333*	2.2333±0.2848*
	TM2	4.1667±0.2728*	3.4667±0.2333*	0.3667±0.0333*	2.4667±0.2333*
	TM3	4.0333±0.1453*	3.3000±0.2646*	0.3667±0.0333*	2.3667±0.2028*

Note: using one-way ANOVA with Tukey test on post-hoc t-tests. * indicates significance ($p<0.05$)

Pearson correlation coefficient *(rho p) on* fetal parameters in chlorpromazine-treated groups.

When a Pearson correlation analysis was done within and across the chlorpromazine-treated groups on the fetal outcomes to establish the correlation significant levels, the strength and the direction of linear relationship on fetal pregnancy outcome, namely:- mean fetal weight, the average length of the crown lump, average head

circumference, average bi-parietal diameter, it showed a strong positive linear relationship and a statistical significance difference ($p \leq 0.01$)between all these variables across all the trimester (TM1, TM2, TM3).

Intra and inter-group Pearson correlational comparison of mean head size/circumference, crown-rump length, average fetal weight, and Mean bi- parietal diameter for the LCPZG, MCPZG, and HCPZG at TM_1, TM2, TM3.

TABLE 1.2

		TM1 FW	TM2 FW	TM3 FW	TM1 CRL	TM2 CRL	TM3 CRL	TM1 HC	TM2 HC	TM3 HC	TM1 BPD	TM2 BPD	TM3 BPD
TM1 FW	R	1	.944**	.943**	.981**	.892**	.875**	.905**	.885**	.892**	.884**	.878**	.929**
	P		.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
TM2 FW	R	.944**	1	.981**	.969**	.971**	.950**	.974**	.970**	.967**	.933**	.941**	.949**
	P	.000		.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
TM3 FW	R	.943**	.981**	1	.974**	.928**	.947**	.950**	.922**	.965**	.919**	.877**	.952**
	P	.000	.000		.000	.000	.000	.000	.000	.000	.000	.000	.000
TM1 CRL	R	.981**	.969**	.974**	1	.915**	.905**	.926**	.909**	.925**	.916**	.914**	.945**
	P	.000	.000	.000		.000	.000	.000	.000	.000	.000	.000	.000
TM2 CRL	R	.892**	.971**	.928**	.915**	1	.918**	.972**	.999**	.936**	.863**	.925**	.862**
	P	.000	.000	.000	.000		.000	.000	.000	.000	.000	.000	.000
TM3 CRL	R	.875**	.950**	.947**	.905**	.918**	1	.943**	.914**	.997**	.863**	.839**	.906**
	P	.000	.000	.000	.000	.000		.000	.000	.000	.000	.001	.000
TM1 HC	R	.905**	.974**	.950**	.926**	.972**	.943**	1	.965**	.955**	.924**	.890**	.930**
	P	.000	.000	.000	.000	.000	.000		.000	.000	.000	.000	.000
TM2 HC	R	.885**	.970**	.922**	.909**	.999**	.914**	.965**	1	.932**	.862**	.935**	.857**
	P	.000	.000	.000	.000	.000	.000	.000		.000	.000	.000	.000
TM3 HC	R	.892**	.967**	.965**	.925**	.936**	.997**	.955**	.932**	1	.884**	.862**	.920**
	P	.000	.000	.000	.000	.000	.000	.000	.000		.000	.000	.000
TM1 BPD	R	.884**	.933**	.919**	.916**	.863**	.863**	.924**	.862**	.884**	1	.900**	.980**
	P	.000	.000	.000	.000	.000	.000	.000	.000	.000		.000	.000
TM2 BPD	R	.878**	.941**	.877**	.914**	.925**	.839**	.890**	.935**	.862**	.900**	1	.873**
	P	.000	.000	.000	.000	.000	.001	.000	.000	.000	.000		.000
TM3 BPD	R	.929**	.949**	.952**	.945**	.862**	.906**	.930**	.857**	.920**	.980**	.873**	1
	P	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	

**Correlation r is significant at the 0.01 level (2-tailed)

P value=0.001 is significant.

IV. DISCUSSION

The study's finding was; the experimental group depicted variation compared to the control group in various parameters. Fetal weight decreased with increasing dose of chlorpromazine, albeit for the low dose where fetal weight markedly increased across all trimesters. When chlorpromazine was administered at different gestation periods, it was noted that the mean fetal weight significantly decreased with increasing dose of chlorpromazine, especially when given during TM1 at a high dose of 3.2000±0.1155 compared to the control 6.633333±0.2403701, $p<0.05$ (table 3.1) these concur with a study carried out by Mohammed and colleagues which showed increased fetal death and reduced fetal weight gain with a high dose of chlorpromazine(Iqbal et al., 2005). However, animals that received low dose chlorpromazine were heavier than the control group across all the trimesters Control 6.633333±0.2403701 LDCPZ TM1 7.4000±0.4041, LDCPZ TM2 7.5333±0.3712, LDCPZ TM3 7.2333±0.1764. These findings followed a study by Platt and others (Patton et al., 2002). Although a previous study was carried out on the use of fluphenazine and diphenylhydantoin, drugs in the same class as chlorpromazine depicted significant weight reduction across all treatment groups as compared to the control (Abdel-Hamid et al., 1996).

Reduction in mean head circumference and bi-parietal diameter were more pronounced when chlorpromazine was administered during TM1 at a high dose. These findings agree with the previous study by Terry and colleagues(Terry et al., 2008). In addition, while Ahmed and colleagues cited an increased risk of congenital malformation such as phocomelia and limb reduction defect with the use of the drug(Ahmed et al.,

2016), Platt and others found that children born of mothers using chlorpromazine and other neuroleptics were taller than non-exposed group(Patton et al., 2002).

V. Conclusion and recommendation

In conclusion, the study established that chlorpromazine, whether given at low or high doses, affects the fetal rat. While a high dose caused significant weight reduction, a low dose caused weight gain, which can negatively affect normal delivery. Therefore, chlorpromazine should be avoided in pregnancy. However, more studies need to be done on higher primates like monkeys to ascertain their safety during pregnancy.

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Declaration of interest

The author declares no conflict of interest.

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