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## Effect of Disulfiram and Copper Sulphate Combination on Pregnancy Rate and Histology of the Uterus in Rats



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### ABSTRACT

This study sought to determine the effect of the drug combination DSF/CuSO<sub>4</sub> on pregnancy rate and histology of the uterus in Wistar rats in a 40 daytime dependent study. Experimental animals were divided into 4 groups of 2 males and 3 females. Group A received (0.2ml distilled water), group B received (0.02ml DMSO), group C received (DSF=18.65mg/kg, CuSO<sub>4</sub>=3.75mg/kg), orally once daily. The results of the study on pregnancy rates and average number of pups littered revealed the following: group A: 33% and 3 pups, group B: 66% and 4 pups, group C: 100% and 11 pups. These results showed a statistically significant increase at ( $p < 0.05$ ) in the rate of pregnancy and number of pups littered by the test groups when compared to the control. Results showed that the histopathology of the uterus of all the groups revealed normal uterine architecture. The study concludes that the drug combination had no deleterious effect on the uterus of the study animals.



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## INTRODUCTION

The incidence of Cancer is of great concern as the morbidity and mortality are on the increase worldwide (American Cancer Society, 2014). Disulfiram (DSF) is used as an aversive therapy of alcoholism. When combined with copper sulphate, there is enhancement of cancer treatment (Georgewill *et al.*, 2015). Disulfiram has promising anticancer effect and is a radiosensitizer (Chen *et al.*, 2001; Cen *et al.*, 2002; Georgewill *et al.*, 2015). Disulfiram bioactivities is beyond its initial clinical application and it is an attractive agent for multiple drug repositioning (Alsaad *et al.*, 2014). One of its most promising traits is the ability to selectively target certain types of cancer, which is reported to be partially affiliated to its ability to form a copper complex that inhibits proteasome function (Skroh, and Cveks., 2012). Disulfiram has been used for many decades in the treatment of alcoholism. (Chen *et al.*, 2013). The mechanism of action includes the induction of free radicals. Disulfiram is reported both as a promising therapeutic compound and as an excellent example of a new class of compounds harnessing copper ions to enhance antibacterial activity (Dalecki *et al.*, 2015). Copper sulphate is widely used chemical compound which is made up of copper, Sulphur and oxygen. Copper sulphate was used in the past as an emetic (Holtzmann *et al.*, 1968). However, copper sulphate is now considered too toxic for this use (Olson *et al.*, 2004) The uterus (womb) is made up of an external layer of smooth muscle known as myometrium, and an internal layer called the endometrium the perimetrium, the outer layer that covers the body of uterus and cervix (Shier *et al.*, 1998).

The uterus is located above the vagina, above and behind the bladder and in front of the rectum. The uterus (womb) accepts fertilized ovum which moves through the utero-tubal junction from the fallopian tube (uterine tube) (Guyton and Hall, 2006).

## METHODOLOGY

The drug combination disulfiram and copper sulphate (DSF and  $\text{CuSO}_4$ ) were administered following the method described by (Georgewill *et al.*, 2015). A total of, 20 rats were used for the study which lasted four Weeks (28 days). Drugs and chemicals were given to the experimental groups while distilled water and DMSO was given to control groups respectively. The Wistar rats were kept in well prepared cages and beddings made of sawdust. The cages were cleaned daily. The females were in proestrus phase. They were randomly divided into four (4) groups of five (5) animals: 2 males and 3 females per group.

The female rats received test drugs (Disulfiram and Copper Sulphate), while the male rats were given only food and water throughout the period of the study. Pregnancy was determined by the presence of sperm in the vaginal fluid and development of permanent diestrus dictated by vaginal smears and observation of physiological bleeding about the 14<sup>th</sup> day. Thereafter, the weights of the rats were measured on day 1, 7, 14 and 21. The activity, rate of pregnancies, deliveries and the size of new pups delivered to the rats of both groups was also measured. On day 28, the animals were sacrificed under diethylether anaesthesia using a desiccator. Uterine tissue was harvested for histopathological analysis.

### **DRUG ADMINISTRATION**

Group A: Received 0.2ml distilled water daily.

Group B: Received 0.02ml DMSO daily

Group C: Received DSF=18.65mg/kg and CuSO<sub>4</sub>= 3.7mg/kg within six (6) hours interval daily throughout the study.

### **ETHICAL CLEARANCE:**

Ethical clearance was obtained from the Ethics committee of University of Port Harcourt.

### **STATISTICAL ANALYSIS**

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) IBM version- 20.0 software One Way Analysis of Variance (ANOVA). The results were considered statistically significant at  $P < 0.05$ . R results were also expressed as percentages.

## RESULTS

**Table 1: Effects of DSF and CuSO<sub>4</sub> combination on pregnancy rates and number of pups littered**

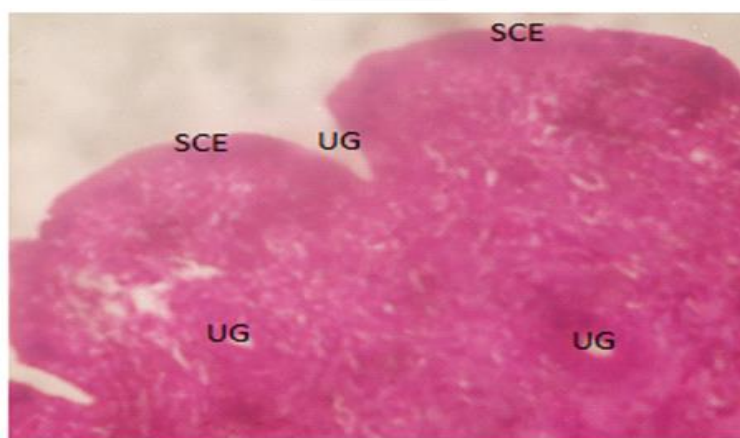
Group	Mean no of pups	Std. Error of Mean	Pregnancy rate (%)	ANOVA
Control	3.6250	0.82	33	0.95
DMSO	4.666	0.55	66	0.84
DSF(18.65mg/kg) +CUSO4(3.75mg/kg)	11.0000	2.65	100	0.022

Differences significant at  $P < 0.05^*$

\*. The mean difference is significant at the 0.05 level.  $N=5$

## HISTOLOGY

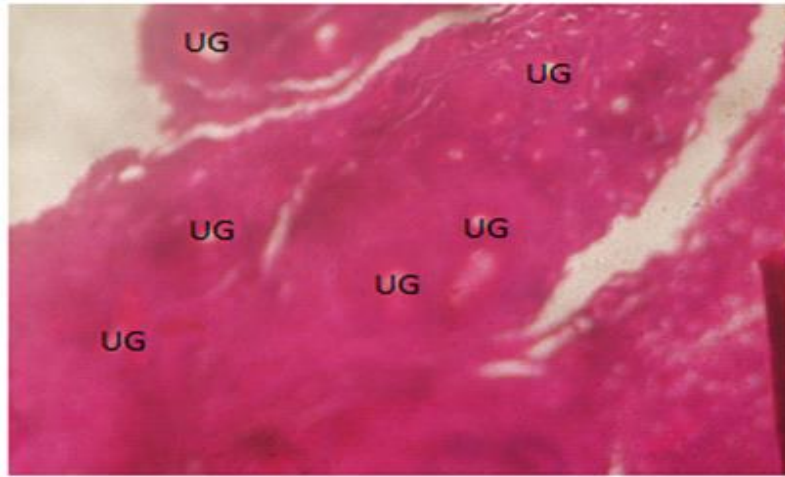
### Effects of Disulfiram and Copper sulphate combination on the histology of the uterus of female Albino Rats



*Mag X400 H&E*

**Fig 1 Control group uterus on day 28 study showing normal architecture;**

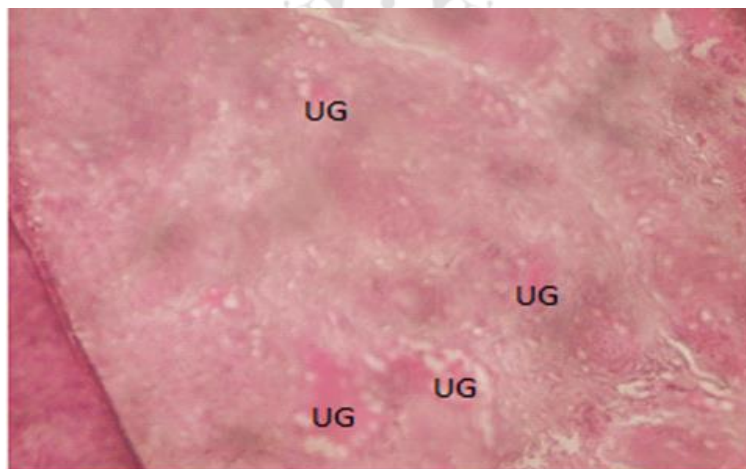
- Uterine glands (UG); lined by simple columnar epithelium. They are patent and tubular.
- Blood Vessels
- The luminal border is lined by Simple Columnar Epithelium (SCE)
- Impression: Histologically normal endometrium in proliferative phase.



*Mag X 400 H&E*

**Fig 2: DMSO group on day28 showing normal architecture;**

- a. Uterine glands (UG); lined by simple columnar epithelium.
- b. Impression: uterine endometrium harvested during proliferative phase of uterine cycle. Histologically normal.



*Mag X 400 H&E*

**Fig 3: Photomicrograph of the Uterus of the female rats treated with low dose of drug combination (DSF=18.65mg/kg and CUSO4=3.74MG/KG), on day28 showing:**

- a. Uterine Gland (UG); Congested and filled with the mucus. Lined by SCE
- b. Impression: histologically normal endometrium in secretory phase.

## DISCUSSION

The dose of disulfiram used in this study was 18.65mg/kg and Copper sulphate was 3.75mg/kg given orally. DMSO was the universal solvent used to dilute disulfiram. The results showed a statistically significant increase in pregnancy rate and number of pups littered by the rats treated with disulfiram and copper sulphate combination when compared with the control (Table 1). The group treated with the drug combination littered higher number of pups than the DMSO and DSLTD groups. This finding agree with that of Amant *et al.* 2015, whose research work stated that chemotherapeutic agents can be administered without an increased risk of fetal distortion and without any damage in neonatal period and early childhood.

The result revealed that there was no statistically significant difference between the control groups and test groups. DMSO is a neutral universal solvent. The uterus of the control group rats revealed normal architecture characterized by uterine gland, lined by simple columnar epithelium as seen in Figure 1. The rats that received DMSO also showed normal endometrium in proliferative phase of the uterus (Fig 2). Photomicrograph of the Uterus of the female rats treated with drug combination (DSF=18.65mg/kg and  $\text{CUSO}_4$ =3.74mg/kg), on day 28 showed the uterine gland congested and filled with the mucus, lined by histologically normal endometrium in secretory phase (Fig 3). The results of this study showed that on day 28 of administration of disulfiram and copper sulphate combination at low dose no deleterious effect was seen on the histology of the experimental rats.

## CONCLUSION

This study concludes that this drug combination at the doses administered showed no deleterious effects on the uterus. Also, the study animals recorded a high rate of pregnancy and increased number of pups which was statistically significant.

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