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Effects of Disulfiram and Copper Sulphate on Hormonal Profile of Female Wistar Rats

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ABSTRACT

This research study evaluated the effects of disulfiram and copper sulphate combination on the hormonal profile of female Wistar rats. Twenty-eight adult female rats were used in this study. The rats were divided into 4 groups of 6 rats per group. The animals received the following: group 1 received 0.2ml of distilled water, group 2 received 0.02ml of DMSO, group 3 received DSF(18.65mg/kg) and $CuS0_4$(3.75mg/kg) and group 4 were given DSF (74.6mg/kg and $CuS0_4$(15mg/kg), orally 6 hours apart daily. Blood samples were collected on days 14, 21, and 28 for assay of female sex hormones. Results of the study revealed that on day 28 for LH group 1: 1.08±0.03, group 2: 0.88±0.03, group 3: 0.96±0.06, and group 4: 1.47±0.65. For FSH group1: 0.42±0.03, group 2: 0.35±0.05, group 3: 0.38±0.02, and group 4: 0.70±0.33. For Prolactin group 1: 1.20±0.00, group 2: 1.25±0.05, group 3: 1.25±0.05, and group 4: 1.25±0.05). For Progesterone group 1: 33.55±0.55, group 2:26.40±21.50, group 3: 24.25±3.45 and group 4: 34.20±13.70. All results obtained for the different parameters were not statistically significant ($P< 0.05$) when compared to the control. This study concludes that the drug combination in the doses used for this study were not deleterious on the reproductive Hormones of female rats.

INTRODUCTION

In the world today, cancer is regarded as a devastating ailment, the effects of cancer to humans have been of a great importance all over the world. Cancer is indeed really a major affliction worldwide. Each year tens of millions of people around the universe are diagnosed of this affliction (cancer) and more than half of them diagnosed die from the effect of cancer. Cancer has or will soon become the number one killer in many parts of the world.

As elderly people are most affected by cancer and population aging continues in many countries (Doll *et al.,* 2005).

The geographical disparity in cancer incidence is largely contributed by factors such as socioeconomic, environmental and lifestyle factors in different regions of the world. Compared with developed countries, developing countries in general may lack the resources to ascertain the current incidence of cancer. (Parkin *et al.,* 2004). Disulfiram is a drug discovered in the 1920s that is used to support the treatment of chronic alcoholism by producing an acute sensitivity to ethanol (alcohol).

Disulfiram works by inhibiting the enzyme acetaldehyde dehydrogenase resulting in the accumulation of acetaldehyde responsible for the unpleasant effects.

Recently, disulfiram is the subject of research for treatment of different cancers (Doyon *et al.,* 2013).

Copper sulphate is a widely used chemical compound which is made up of copper, Sulphur and oxygen. Copper sulphate was used in the past as an emetic. However, copper sulphate is now considered too toxic for this use (Olson *et al.,* 2004).

An example of potential drug repurposing is disulfiram (DSF). Many studies to evaluate the potency of disulfiram against certain types of cancer (Robinson *et al.,* 2013, Triscott *et al.,* 2012), are ongoing.

The ovary is the primary source of sex hormones in women during the childbearing years (between puberty and menopause). When the sex hormones are well regulated by the follicle stimulating hormone (FSH) and Luteinizing hormone (LH) from the pituitary, then the normal events occur in menstrual cycle which includes maturation of follicle in ovary, secretion increase in estrogen, ovum release (Ketzung; 2004). The hormones controlling the

female reproductive system include gonadotropin-releasing hormone (GnRH), follicle, stimulating hormone (FSH) and luteinizing hormone (LH), all of which are produced in the brain; Estrogen and progesterone produced by the ovaries and the corpus luteum; and human chorionic gonadotropin.

METHODOLOGY

EXPERIMENTAL ANIMALS

Female Wistar rats weighing between 200-260 g were used for this study. They were obtained from the animal house of Pharmacology Department, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The rats were kept at room temperature and a 12:12 h light/dark cycle with free access to food maintained. They were fed with standard pelletized feed (manufactured by Premier Feed Mill Co. Ltd) and water *ad libitum*. The rats used were handled based on the guidelines of National Institute Health guide for care and use of laboratory animals (Pub No. 85-23 revised 1985).

DRUG ADMINISTRATION

The drug combinations disulfiram and copper sulphate (DSF and $CuSO_4$) were administered following the method described by (Georgewill *et al.*, 2015). Disulfiram was manufactured by SHIJIAZFUANGAOPHARM CO. Imported from Japan to Nigeria. Copper Sulphate was from Fisher Scientific Company, Chemical manufacturing Division, Fair Lawn, New Jersey 07410 made in U.S.A. The universal solvent was Dimethyl sulfoxide (DMSO), and distilled water for control.

A total of Twenty- Four Wistar rats were used for this study and were randomly divided into four (4) groups of six (6) animals each.

Group 1: 0.2 ml distilled water only.

Group 2: 0.02 ml DMSO once daily.

Group 3: DSF=18.65mg/kg and $CuSO_4$= 3.7mg/kg within six (6) hours interval once daily.

Group 4: DSF=74.60mg/kg and $CuSO_4$= 15mg/kg within six (6) hours interval once daily.

SAMPLE COLLECTION

The blood sample was collected on days 14, 21 and 28 for Hormonal assay.

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 $P< 0.05$.

RESULTS

Table 1: Effects of DSF and $CuS0_4$ combination treatment on serum Luteinizing Hormone (LH) in female Wister rats.

GROUP	TREATMENT	DURATION OF TREATMENT (DAYS)		
		LH (IU/L) Day 14	LH (IU/L) Day 21	LH (IU/L) Day 28
1	DSTLD	0.83±0.02	0.95±0.07	1.08±0.03
2	DMSO	0.97±0.07	1.25±0.27	0.88±0.03
3	DSF(18.65mg/kg) + CUS04(3.75mg/kg)	0.93±0.03	0.87±0.02	0.96±0.06
4	DSF(74.60mg/kg) + $CUS0_4$(15mg/kg)	1.16±0.36	1.53±0.48	1.47±0.65

Differences not significant ($P>0.05$)

- DSTLD= Distilled water
- DMSO= Dimethyl sulfoxide
- DSF= Disulfiram
- $CuS0_4$= Copper sulphate

Table 2: Effects of DSF and $CuSO_4$ combination treatment on serum Follicle Stimulating Hormone (FSH) in female rats

GROUP	TREATMENT	DURATION OF TREATMENT (DAYS)		
		FSH (IU/L) Day 14	FSH (IU/L) Day 21	FSH (IU/L) Day 28
1	DSTLD	0.27±0.03	0.31±0.09	0.42±0.03
2	DMSO	0.37±0.07	0.47±0.06	0.35±0.05
3	DSF(18.65mg/kg) + $CUS0_4$(3.75mg/kg)	0.36±0.04	0.36±0.01	0.38±0.02
4	DSF(74.60mg/kg) + CUS04(15mg/kg)	0.50±0.19	0.58±0.19	0.70±0.33

Difference not significant (P>0.05)

- DSTLD= Distilled water
- DMSO= Dimethyl sulfoxide
- DSF= Disulfiram
- $CuS0_4$= Copper sulphate
- N= 6

Table 3: Effects of DSF and $CuSO_4$ combination treatment on serum Progesterone Hormone in female rats

Group	TREATMENT	DURATION OF TREATMENT		
		Progesterone (ng/ml) Day 14	Progesterone (ng/ml) Day 21	Progesterone (ng/ml) Day 28
1	DSTLD	20.30±1.2	22.95±3.45	33.55±0.55
2	DMSO	25.35±10.35	31.50±11.30	26.40±21.50
3	DSF(18.65mg/kg) +$CUS0_4$(3.75mg/kg)	29.05±8.05	20.95±0.55	24.25±3.45
4	DSF(74.60mg/kg) + CUS04(15mg/kg)	24.25±0.05	30.05±4.05	34.20±13.70

Differences not significant (P>0.05)

- DSTLD= Distilled water
- DMSO= Dimethyl sulfoxide
- DSF= Disulfiram
- CuS04= Copper sulphate

Table 4: Effects of DSF and $CuSO_4$ combination treatment on serum Prolactin Hormone in female rats

GROUP	TREATMENT	DURATION OF TREATMENT (DAYS)		
		Prolactin (ng/ml) Day 14	Prolactin (ng/ml) Day 21	Prolactin (ng/ml) Day 28
1	DSTLD	1.30±0.00	1.30±0.00	1.20±0.00
2	DMSO	1.15±0.05	1.25±0.05	1.25±0.05
3	DSF(18.65mg/kg) +CUS04(3.75mg/kg)	1.25±0.05	1.20±0.00	1.25±0.05
4	DSF(74.60mg/kg) + $CUS0_4$(15mg/kg)	1.35±0.05	1.30±0.10	1.25±0.05

Difference not significant (P>0.05)

- DSTLD= Distilled water
- DMSO= Dimethyl sulfoxide
- DSF= Disulfiram
- $CuS0_4$= Copper sulphate

DISCUSSION

Establishment of a new drug is expensive and time consuming. However, evaluating new functions of already existing therapeutic agents has attracted researcher's attention due to the low and fast track (called repositioning or repurposing). DSF is an aldehyde dehydrogenase inhibitor and Copper chelator has been revealed for treatment of alcoholism since 1920s Johansson; 2008. This research work evaluated the effects of Disulfiram and Copper Sulphate on the hormonal profile of Female Wistar rats. It showed that Disulfiram and Copper

Sulphate combination at Low Dose (LD) DSF = 18.65 mg/kg/ $CuSO_4$=3.75 mg/kg had no statistically significant effect on the levels of LH. Also, rats given High Dose (HD) Disulfiram=74.6mg/kg and copper sulphate=15mg/kg showed no statistically significant effect (Table 1). This implies that the combination of DSF and $CuSO_4$ combination at high dose after 14 days did not produce any adverse effect on the serum LH of the test group when compared with the control group. Similarly, on day 21 there was no significant decrease in the levels of LH, though there was a slight decrease in high dose group, but this was not statistically significant when compared with the control groups (Table 1). This is in agreement with Georgewill *et al.* 2015 whose work revealed that DSF and $CuSO_4$ combination at low dose after 90 days did not produce significant effect on LH levels. On day 28 of this study, the level of the LH showed a decrease in the high dose but the decrease was not statistically significant when compared with the control groups (Table 1). However, the American Cancer Society (ACS) revealed that most cancers can be treated with chemotherapy during pregnancy at the second or third trimester, and also that some types of cancer like Cervical, Breast and Ovarian cancers are the most common cancers treated with pregnancy during the first trimester, and does not seem to harm the offspring (American Cancer Society; 2012). This is in line the current research evaluating the effects on the hormonal profile of female Wistar rats which revealed no deleterious effects on the rats treated (Disulfiram and Copper sulphate combination). Similarly, Disulfiram = 18.65mg/kg and Copper Sulphate = 3.75mg/kg on FSH levels showed no significant effect on day 14, 21 and day 28 respectively (Table 2). On progesterone levels, disulfiram and copper Sulphate (DSF & $CUSO_4$) combination at doses of DSF =18.65mg/kg and $CUSO_4$ =3.75mg/kg / 74.6mg/kg and 15mg/kg showed no significant difference in the level of progesterone when compared with the control groups. The high dose of the drug combination on day 21 and 28 showed slight decrease but the decrease was not statistically significant when compared with the control group (Table 3). Low dose of disulfiram and copper sulphate combination effect on Prolactin shows that there was no significant effect on day 14, day 21 and day 28 of the study. For the group given high dose, there was no statistically significant effect seen(Table 4). This finding suggests a good safety profile of the combination on serum Prolactin hormone at the doses studied.

CONCLUSION

The result of this study shows that DSF and $CuSO_4$ combination at the doses studied did not show any significant deleterious effects on the reproductive hormones such as Luteinizing Hormone, Follicle Stimulating Hormone, Progesterone and Prolactin of female albino rats. However, the researchers believe caution should still be applied in the use of this drug combination.

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