

EFFECT OF DISULFIRAM/COPPER GLUCONATE COMBINATION ON HEPATIC FUNCTION AND BLOOD CHOLESTEROL LEVELS IN RATS

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ABSTRACT

This study sought to determine the chronic toxicological effects of the disulfiram/copper gluconate drug combination in rodents in a 90 day dose and time dependent study. A total of 88 rats weighing between 260-300g were used. The rats were divided into eleven groups consisting of 8 rats each with Groups 1 and 2 serving as control groups. The control groups received normal saline as placebo and 99.5% dimethyl sulfoxide (DMSO) (Solvent control). The drugs were administered as 1/5th, 1/10th and 1/20th of the LD₅₀ of 373mg/kg and 75mg/kg for disulfiram and copper gluconate respectively. Dosing was done daily with that of the combination given 12hours apart. Blood samples were collected via cardiac puncture in heparinised bottles, centrifuged and the serum decanted on days 30,45,60 and 90 for analysis. Hepatic function parameters showed a significantly (P < 0.05) dose and time dependent increase in the liver enzymes (AST,ASP,ALP) and a significant(P<0.05) decrease in total protein. It concluded that the DSF/CG combination showed a synergistic dose dependent hepatotoxicity.

INTRODUCTION

The search for cheaper, readily available drugs for use in cancer chemotherapy has fuelled the need to repurpose old drugs no longer in use for their original intention.

Repurposing drugs is not novel as several examples abound. Two failed cancer drugs which have been repurposed include zidovudine (AZT), the first antiviral approved for HIV/AIDS in 1987 and farnesyltransferase inhibitor (FTI) which was recently used to successfully treat children with the rapid-aging disease Progeria in a 2012 clinical trial. Other drugs that have been repurposed for other indications include raloxifene which was initially developed to treat osteoporosis but was approved in 2007 for invasive breast cancer in post-menopausal women. Disulfiram (antabuse) and copper gluconate are also undergoing research for possible use in cancer chemotherapy. A number of researchers have worked on cancer cell lines (Chen *et al*, 2011, Grossmann *et al*, 2011, papaioannou *et al*, 2013) have suggested that based on the apoptotic effect of the drug combination observed in cancer cell lines, clinical trials could be undertaken. This research study therefore set out to assess the toxicological implications of repurposing disulfiram and copper gluconate for chronic use in cancer therapy.

METHODOLOGY

88 Albino Swiss rats of both sexes weighing between 260g- 300g obtained from the department of Pharmacology animal house were used for this study. The rats were bred and maintained under suitable conditions, allowed an acclimatization period of two (2) weeks, housed in hygienic cages in groups of five and allowed free access to feed obtained from

vital feeds UAC PLC and water *ad libitum*. The beddings were changed and cages cleaned out on alternate days. Animals were handled according to Helsinki declaration on animal care. The animals were divided into 11 groups, each consisting of 8 rats each. The groups included those for treatment and the control groups. Drugs were administered orally via a 1ml syringe.

CHRONIC TOXICITY TESTS

This study spanned 3 months and was domiciled in the Department of Pharmacology, University of Port Harcourt, Animal house and Laboratory. A dose and time dependent toxicological evaluation of the effects of these individual drugs and their combinations on the liver function of rats was evaluated. The rats were divided into eleven groups consisting of 8 rats each. Groups 1 and 2 served as control groups and the rats received normal saline as placebo and 99.5% DMSO (Solvent control) respectively. Drugs were administered orally via a 1ml syringe.

The test group rats were divided into groups 3, 4 and 5 consisting of 24 rats in each group. Drug administration was done orally for 90 days as follows;

Control group 1 rats received 1ml of normal saline orally daily for 90 days

Solvent control group 2 received 0.5ml of DMSO orally daily for 90 days

Group 3a rats received *15mg/kg of copper gluconate daily orally

Group 3b rats received *7.5mg/kg of copper gluconate daily orally

Group 3c rats received *3.75mg/kg of copper gluconate daily orally

*doses were 1/5th, 1/10th and 1/20th of the LD₅₀ of Copper gluconate

Group 4a rats received °74.6mg/kg of DSF and *15mg/kg of copper gluconate daily orally

Group 4b rats received °37.3mg/kg of DSF and *7.5mg/kg of copper gluconate daily orally

Group 4c rats received °18.65mg/kg of DSF and *3.75mg/kg of copper gluconate daily orally

° Doses were 1/5th, 1/10th and 1/20th of the LD₅₀ of disulfiram (DSF)

* Doses were 1/5th, 1/10th and 1/20th of the LD₅₀ of copper gluconate

N/B The drug combination was given following the protocol of Grossman *et al*, 2011 .

Group 5a rats received °74.6mg/kg of DSF daily orally

Group 5b rats received °37.3mg/kg of DSF daily orally

Group 5c rats received °18.65mg/kg of DSF daily orally

° Doses were 1/5th, 1/10th and 1/20th of the LD₅₀ of disulfiram (DSF)

COLLECTION OF SAMPLES

Two animals per group were sacrificed using diethyl ether anaesthesia and blood samples were obtained on days 30, 45, 60 and 90 for analysis via cardiac puncture. Blood samples were centrifuged at 3000rpm for 15minutes and serum separated from the cells. The samples were then assayed for ALT,AST, ALP using commercially available test kits by Randox Laboratories, UK. Total protein levels were assayed using the Biuret 1857 method was adopted

STOCK SOLUTIONS; Were prepared from 99.5% DMSO for disulfiram and distilled water for copper gluconate. Pure analytical grade samples, CAS No. 527-09-3(98% min purity) and CAS No. 97-77-8(98% min purity) obtained from Shijiazhuang Aopharm Import and Export Co. Limited China were used for the study.

RESULTS:

Table 4.4ai: The Effect of Low Dose DSF, CG and DSF/CG Combination on AST

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	75.00 ± 2.89	75.00 ± 1.73	76.67 ± 3.33	75.33 ± 0.67
Control 2	73.00 ± 5.00	70.67 ± 0.67	72.00 ± 3.00	74.67 ± 1.76
DSF (18.65mg/kg)	76.33 ± 2.96	78.67 ± 0.88	79.00 ± 1.16	80.00 ± 2.00
CG (3.75mg/kg)	89.00 ± 4.00*	82.00 ± 1.16*	85.00 ± 1.73*	92.33 ± 1.45*
DSF/CG (18.65/3.75mg/kg)	104.7 ± 2.03*	91.17 ± 0.93*	93.33 ± 1.67*	108.30 ± 7.27*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=3

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as µ/l

Table 4.4aii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on AST

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	58.00 ± 2.08	59.33 ± 0.67	59.33 ± 4.70	59.00 ± 3.22
Control 2	55.67 ± 1.20	58.67 ± 0.67	57.07 ± 5.04	56.00 ± 1.00
DSF (37.3mg/kg)	72.33 ± 1.45 *	72.33 ± 1.45 *	75.00 ± 1.16 *	80.00 ± 2.89 *
CG (7.5mg/kg)	81.67 ± 3.67 *	82.67 ± 4.67 *	89.33 ± 1.33 *	93.33 ± 2.40 *
DSF/CG (37.3/7.5mg/kg)	90.67 ± 6.36 *	90.67 ± 6.36 *	98.00 ± 6.00 *	120.0 ± 11.55*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as µ/l

Table 4.4aiii: Effect of High Dose DSF, CG and DSF/CG Combination on AST

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	59.00 ± 3.22	59.00 ± 2.08	61.67 ± 1.67	65.00 ± 2.89
Control 2	56.00 ± 1.00	58.00 ± 1.16	59.33 ± 0.66	58.67 ± 0.67
DSF (74.6mg/kg)	81.67 ± 1.67 *	85.00 ± 2.89 *	86.67 ± 4.41 *	90.00 ± 2.89 *
CG (15mg/kg)	96.67 ± 2.40 *	97.33 ± 2.57 *	98.33 ± 2.67 *	99.33 ± 2.67 *
DSF/CG (74.6/15mg/kg)	112.3 ± 5.36 *	111.7 ± 6.01 *	111.7 ± 6.01 *	125.0 ± 7.64 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as µ/l

Table 4.4bi: The Effect of Low dose DSF, CG and DSF/CG Combination on ALT

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	58.00 ± 2.00	62.33 ± 1.45	68.00 ± 3.06	65.67 ± 5.36
Control 2	52.67 ± 1.33	61.67 ± 3.33	58.00 ± 2.00	62.67 ± 4.37
DSF (18.65mg/kg)	60.67 ± 0.88	67.17 ± 0.60*	76.00 ± 1.00*	85.67 ± 4.70*
CG (3.75mg/kg)	61.00 ± 1.00	72.00 ± 1.73*	88.33 ± 4.41*	87.67 ± 5.78*
DSF/CG (18.65/3.75mg/kg)	62.50 ± 1.32	90.17 ± 2.89*	98.33 ± 4.41*	102.30 ± 1.45*

Results are expressed as mean \pm SEM, the superscript (*) means significant difference with respect to control at $p < 0.05$ (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as μ /l

Table 4.4bii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on ALT

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	52.00 \pm 2.00	56.33 \pm 1.86	57.00 \pm 4.93	58.67 \pm 1.86
Control 2	54.33 \pm 2.333	54.33 \pm 2.333	59.67 \pm 2.728	56.33 \pm 0.882
DSF (37.3mg/kg)	65.00 \pm 2.00 *	67.00 \pm 2.00 *	68.67 \pm 1.33 *	70.67 \pm 1.13 *
CG (7.5mg/kg)	71.00 \pm 1.00 *	72.33 \pm 2.33 *	74.33 \pm 2.33*	79.33 \pm 6.77 *
DSF/CG (37.3/7.5mg/kg)	90.00 \pm 4.93 *	95.67 \pm 11.02 *	97.67 \pm 1.02 *	111.00 \pm 6.66 *

Results are expressed as mean \pm SEM, the superscript (*) means significant difference with respect to control at $p < 0.05$ (ANOVA).n =5

KEY: Control 1- Distilled water

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as millimols per litre (mmol/l)

Table 4.4biii: The Effect of High Dose DSF, CG and DSF/CG Combination on ALT

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	53.67 \pm 1.86	55.00 \pm 1.73	58.67 \pm 0.67	62.00 \pm 2.31
Control 2	56.33 \pm 0.88	57.33 \pm 2.33	57.67 \pm 1.45	57.00 \pm 1.00
DSF (74.6mg/kg)	69.67 \pm 2.33 *	73.00 \pm 1.00 *	77.33 \pm 3.93 *	81.00 \pm 4.58*
CG (15mg/kg)	87.33 \pm 3.71 *	89.00 \pm 4.58 *	90.67 \pm 5.81 *	91.67 \pm 5.91*
DSF/CG (74.6mg/kg)	102.7 \pm 3.71 *	105.0 \pm 1.73 *	106.7 \pm 1.67 *	126.7 \pm 8.82*

Results are expressed as mean \pm SEM, the superscript (*) means significant difference with respect to control at $p < 0.05$ (ANOVA).n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as μ /l

Table 4.4ci: The Effect of Low Dose DSF, CG and DSF/CG Combination on ALP

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	43.33 \pm 1.67	44.33 \pm 1.27	47.67 \pm 1.45	45.00 \pm 2.89
Control 2	39.33 \pm 0.67	38.67 \pm 0.66	44.33 \pm 2.33	40.67 \pm 1.76
DSF (18.65mg/kg)	44.33 \pm 0.67	45.33 \pm 2.96	46.67 \pm 6.01	47.67 \pm 11.26
CG (3.75mg/kg)	51.67 \pm 1.45 *	61.00 \pm 2.08 *	65.33 \pm 4.41 *	68.33 \pm 6.67 *
DSF/CG (18.65/3.75mg/kg)	58.00 \pm 1.16 *	70.67 \pm 6.44 *	88.67 \pm 5.93 *	101.70 \pm 6.14 *

Results are expressed as mean \pm SEM, the superscript (*) means significant difference with respect to control at $p < 0.05$ (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as μ /l

Table 4.4cii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on ALP

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	48.33 ± 4.41	50.00 ± 5.77	50.33 ± 3.93	52.67 ± 2.33
Control 2	46.67 ± 1.667	49.00 ± 4.509	28.00 ± 1.528	50.02 ± 1.23
DSF (37.3mg/kg)	50.33 ± 4.71	52.33 ± 4.91	62.33 ± 4.91 *	65.34 ± 4.81 *
CG (7.5mg/kg)	76.00 ± 6.51 *	78.00 ± 6.31 *	79.00 ± 6.50 *	82.00 ± 5.69 *
DSF/CG (37.3/7.5mg/kg)	89.67 ± 2.91*	91.00 ± 4.58 *	93.00 ± 4.68 *	101.0 ± 2.08 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as µ/l

Table 4.4ciii: The Effect of High Dose DSF, CG and DSF/CG Combination on ALP

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	42.67 ± 2.33	46.67 ± 1.67	52.33 ± 4.33	56.33 ± 2.96
Control 2	41.33 ± 1.856	45.20 ± 1.08	51.00 ± 2.25	54.22 ± 2.00
DSF (74.6mg/kg)	63.33 ± 4.41 *	75.00 ± 8.66 *	81.67 ± 6.01 *	80.00 ± 5.77 *
CG (15mg/kg)	77.00 ± 6.03 *	83.67 ± 5.84 *	88.00 ± 1.53 *	91.00 ± 3.79 *
DSF/CG (74.6/15mg/kg)	98.33 ± 2.03 *	99.00 ± 2.08 *	102.70 ± 2.67*	111.70 ± 4.41*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as µ/l

Table 4.4di: The Effect of Low Dose DSF, CG and DSF/CG Combination on Total protein

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	73.16 ± 0.02	73.14 ± 0.02	73.16 ± 0.02	73.17 ± 0.01
Control 2	72.00 ± 0.10	71.00 ± 0.21	70.05 ± 0.03	72.12 ± 0.23
DSF (18.65mg/kg)	63.07 ± 0.01*	63.07 ± 0.01*	53.07 ± 0.01*	43.06 ± 0.01*
CG (3.75mg/kg)	63.00 ± 0.03*	63.03 ± 0.04*	53.01 ± 0.03*	43.00 ± 0.03*
DSF/CG (18.65/3.75mg/kg)	63.06 ± 0.03*	63.08 ± 0.02*	53.08 ± 0.02*	42.06 ± 0.03*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as g/l

Table 4.4di: The Effect of Low Dose DSF, CG and DSF/CG Combination on Total protein

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	73.16 ± 0.02	73.14 ± 0.02	73.16 ± 0.02	73.17 ± 0.01
Control 2	72.00 ± 0.10	71.00 ± 0.21	70.05 ± 0.03	72.12 ± 0.23
DSF (18.65mg/kg)	63.07 ± 0.01*	63.07 ± 0.01*	53.07 ± 0.01*	43.06 ± 0.01*
CG (3.75mg/kg)	63.00 ± 0.03*	63.03 ± 0.04*	53.01 ± 0.03*	43.00 ± 0.03*
DSF/CG (18.65/3.75mg/kg)	63.06 ± 0.03*	63.08 ± 0.02*	53.08 ± 0.02*	42.06 ± 0.03*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram
CG- Copper gluconate
*All results are expressed as g/l

Table 4.4dii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on Total protein

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	73.18 ± 0.02	73.17 ± 0.02	73.16 ± 0.02	73.16 ± 0.02
Control 2	72.05 ± 0.12	73.00 ± 0.01	72.10 ± 0.01	73.06 ± 0.03
DSF (37.3mg/kg)	62.97 ± 0.06*	62.88 ± 0.07*	52.88 ± 0.07*	42.72 ± 0.10*
CG (7.5mg/kg)	62.94 ± 0.07*	62.95 ± 0.06*	53.000 ± 0.03*	42.48 ± 0.16*
DSF/CG (37.3/7.5mg/kg)	62.77 ± 0.04*	62.74 ± 0.01*	52.50 ± 0.19*	42.28 ± 0.22*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as g/l

Table 4.4diii: The Effect of High Dose DSF, CG and DSF/CG Combination on Total protein

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	73.17 ± 0.03	73.17 ± 0.02	73.17 ± 0.01	73.17 ± 0.02
Control 2	70.15 ± 0.02	72.50 ± 0.03	72.80 ± 0.02	73.00 ± 0.15
DSF (74.6mg/kg)	62.49 ± 0.02*	62.37 ± 0.08*	52.06 ± 0.10*	41.98 ± 0.03*
CG (15mg/kg)	62.38 ± 0.10*	62.15 ± 0.03*	52.06 ± 0.07*	41.54 ± 0.05*
DSF/CG (74.6/15mg/kg)	62.12 ± 0.12*	62.06 ± 0.15*	51.84 ± 0.02*	40.03 ± 0.28*

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as g/l

Table 4.5ai: The Effect of Low Dose DSF, CG and DSF/CG Combination on Triglycerides

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	71.00 ± 0.58	74.33 ± 0.86	76.33 ± 0.41	79.00 ± 0.30
Control 2	75.00 ± 22.55	81.67 ± 20.88	74.55 ± 1.56	76.32 ± 0.96
DSF (18.65mg/kg)	74.33 ± 1.86	132.70 ± 1.20 *	133.00 ± 1.16 *	134.70 ± 0.67 *
CG (3.75mg/kg)	71.67 ± 4.91	133.70 ± 1.45 *	134.30 ± 0.88 *	135.30 ± 0.88 *
DSF/CG (18.65/3.75mg/kg)	72.00 ± 1.73	136.30 ± 0.88 *	138.00 ± 1.16 *	139.70 ± 0.88 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as mmol/l

Table 4.5aii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on Triglycerides

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	86.7 ± 1.67	86.67 ± 1.64	78.33 ± 1.78	85.00 ± 0.21
Control 2	83.33 ± 0.21	85.33 ± 0.21	86.67 ± 0.88	87.67 ± 0.98
DSF (37.3mg/kg)	134.7 ± 0.67 *	136.70 ± 0.67 *	138.00 ± 1.16 *	141.70 ± 1.67 *
CG (7.5mg/kg)	135.0 ± 1.16 *	136.70 ± 0.33 *	137.70 ± 0.33 *	141.30 ± 0.67 *
DSF/CG (37.3/7.5mg/kg)	139.7 ± 0.88 *	140.30 ± 0.23 *	141.70 ± 0.33 *	142.30 ± 1.45 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as mmol/l

Table 4.5aiii: The Effect of High Dose DSF, CG and DSF/CG Combination on Triglycerides

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	84.00 ± 1.01	86.67 ± 2.28	85.67 ± 2.46	89.33 ± 1.02
Control 2	85.00 ± 2.55	81.67 ± 2.88	82.67 ± 2.85	83.33 ± 2.21
DSF (74.6mg/kg)	139.00 ± 2.08 *	145.00 ± 1.73 *	145.00 ± 2.89 *	147.70 ± 2.73 *
CG (15mg/kg)	136.70 ± 1.67 *	144.70 ± 1.76 *	145.70 ± 3.48 *	146.30 ± 1.67 *
DSF/CG (74.6/15mg/kg)	135.70 ± 3.48 *	146.00 ± 1.00 *	147.00 ± 1.53 *	150.70 ± 1.76 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as mmol/l

Table 4.5bi: The Effect of Low Dose DSF, CG, and DSF/CG Combination on HDL-Chol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	51.00 ± 3.22	54.33 ± 1.20	54.33 ± 1.20	62.33 ± 8.88
Control 2	50.67 ± 0.66	51.67 ± 0.67	52.07 ± 0.07	52.17 ± 0.77
DSF (18.65mg/kg)	50.00 ± 2.89	37.33 ± 2.67 *	36.67 ± 2.40 *	35.00 ± 3.06 *
CG (7.5mg/kg)	32.67 ± 0.67 *	32.00 ± 1.16 *	31.33 ± 1.76 *	30.67 ± 1.76 *
DSF/CG (18.65/.5mg/kg)	31.17 ± 0.60 *	29.33 ± 0.67 *	28.00 ± 1.16 *	26.67 ± 1.76 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p≤0.05 (ANOVA).n=3

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as mmol/l

Table 4.4bii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on HDL-Chol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	64.00 ± 1.83	64.00 ± 3.33	64.00 ± 1.42	71.00 ± 1.67
Control 2	56.67 ± 0.67	58.00 ± 2.887	60.67 ± 2.90	66.00 ± 2.05
DSF (37.3mg/kg)	32.33 ± 1.20 *	31.33 ± 1.76 *	30.33 ± 2.73 *	29.67 ± 2.33 *
CG (7.5mg/kg)	30.00 ± 2.00 *	29.33 ± 1.33 *	27.33 ± 2.33 *	26.33 ± 2.96 *
DSF/CG (37.3/7.5mg/kg)	24.33 ± 0.33 *	23.33 ± 1.33 *	22.33 ± 1.43 *	21.67 ± 1.76 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n= 5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as mmol/l

Table 4.4biii: The Effect of High Dose DSF, CG and DSF/CG Combination on HDL-Chol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	68.33 ± 1.01	75.00 ± 1.02	75.00 ± 1.10	75.00 ± 1.11
Control 2	64.00 ± 1.33	66.00 ± 1.27	68.00 ± 1.31	71.00 ± 1.58
DSF (74.6mg/kg)	28.67 ± 2.40 *	27.67 ± 2.40 *	26.33 ± 2.67 *	25.33 ± 2.67 *
CG (15mg/kg)	25.67 ± 2.33 *	22.00 ± 2.00 *	21.33 ± 2.40 *	20.00 ± 1.16 *
DSF/CG (74.6/15mg/kg)	21.67 ± 1.20 *	20.33 ± 1.20 *	18.67 ± 0.67 *	16.33 ± 0.88 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=3

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as mmol/l

Table 4.5ci: The Effect of Low Dose DSF, CG and DSF/CG Combination on Total Cholesterol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	65.00 ± 1.41	66.33 ± 1.77	67.33 ± 1.67	77.33 ± 1.99
Control 2	61.67 ± 1.03	63.67 ± 1.95	65.67 ± 1.91	67.67 ± 0.93
DSF (18.65mg/kg)	81.00 ± 0.58*	81.33 ± 0.67*	121.0 ± 0.58 *	131.7 ± 4.41 *
CG (3.75mg/kg)	83.33 ± 1.20*	85.33 ± 1.20*	126.7 ± 0.88 *	131.0 ± 2.08 *
DSF/CG (18.65/3.75mg/kg)	84.33 ± 2.33*	86.00 ± 1.00*	128.7 ± 11.86 *	133.7 ± 3.28 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

Control 2- DMSO

DSF- Disulfiram

CG- Copper gluconate

*All results are expressed as mmol/l

Table 4.5cii: The Effect of Medium Dose DSF, CG and DSF/CG Combination on Total Cholesterol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	77.33 ± 1.99	84.00 ± 1.01	82.33 ± 1.68	85.00 ± 1.21
Control 2	71.67 ± 1.70	76.67 ± 1.40	78.00 ± 1.11	80.00 ± 1.21
DSF (37.3mg/kg)	134.00 ± 7.02 *	138.70 ± 3.53 *	136.70 ± 3.53 *	140.30 ± 1.67 *
CG (7.5mg/kg)	135.70 ± 2.33 *	135.70 ± 4.70 *	141.00 ± 2.08 *	143.00 ± 1.00 *
DSF/CG (37.3/7.5mg/kg)	136.70 ± 3.28 *	138.00 ± 3.06 *	141.30 ± 0.67 *	142.30 ± 1.45 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1 – Distilled water

Control 2- DMSO

DSF- Disulfiram

CG - Copper gluconate

*All results are expressed as mmol/l

Table 4.5ciii: The Effect of High Dose DSF, CG and DSF/CG Combination on Total Cholesterol

TREATMENT GROUPS	DURATION OF TREATMENT			
	30 Days	45 Days	60 Days	90 Days
Control 1	90.67 ± 1.51	92.57 ± 1.59	94.77 ± 10.59	95.67 ± 10.59
Control 2	80.00 ± 25.17	85.00 ± 20.21	85.00 ± 20.21	85.00 ± 20.21
DSF (74.6mg/kg)	135.0 ± 2.89 *	131.7 ± 1.67 *	133.3 ± 1.67 *	135.0 ± 2.89 *
CG (15mg/kg)	138.3 ± 5.24 *	139.0 ± 4.58 *	140.7 ± 5.81 *	145.7 ± 2.33 *
DSF/CG (74.6/15mg/kg)	144.0 ± 6.11 *	148.0 ± 4.16 *	154.0 ± 2.31 *	156.7 ± 1.33 *

Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to control at p<0.05 (ANOVA). n=5

KEY: Control 1- Distilled water

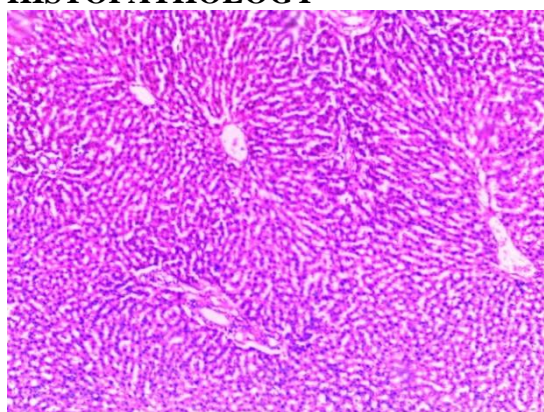
Control 2- DMSO

DSF- Disulfiram

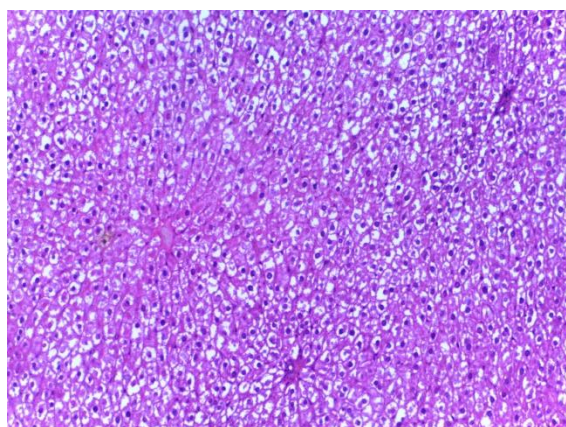
CG- Copper gluconate

*All results are expressed as mmol/l

HISTOPATHOLOGY



A: control showing normal cellular architecture with prominent non congested central veins and cord of hepatocytes



D: At 90 days. Photomicrograph shows generalised severe cytoplasmic vacuolations

DISCUSSION

AST, also known as serum glutamic oxaloacetic transaminase or aspartate amino transferase is raised in acute liver damage. It is also present in cardiac and skeletal muscles and red blood cells so it is not specific to the liver. The ratio of AST to ALT can be used to differentiate between causes of liver damage (Nyblom *et al*, 2004). AST/ALT elevations instead of ALP elevations favour liver cell necrosis as a mechanism over cholestasis. When AST and ALT are greater than three times normal but not greater than 1000 iu/l, differentials could include alcohol toxicity, viral hepatitis, drug-induced liver necrosis, liver cancer, sepsis etc. ALT is a

key metabolic enzyme released in damaged hepatocytes. An increase in ALT could be indicative of an adaptive response to leakage into the blood stream due to metal toxicity (Adaramoye *et al*, 2008). Osuola *et al*, (2014) in their paper, opine that since ALT also plays a part in transforming protein to glycogen, which is the major reserve fuel of the body during stress-induced toxicity in the liver, exposure to heavy metals induces stress damage of tissues and organs and this will occur with elevation and liberation of ALT into the circulation. ALT is known to increase only in heavy metal poisoning, toxic hepatitis and muscular dystrophy and that it is increased more significantly than AST in heavy metal exposure indicative of liver damage (Nduka, 1999, Shalan *et al*, 2005). ALP is an enzyme in the cell lining of the biliary ducts of the liver and its activity increases in hepatocellular damage, as it is a marker enzyme of damage for the plasma membrane and endoplasmic reticulum (Humann *et al*, 2001, Shahjahan *et al*, 2004).

The mechanism by which chronic disulfiram therapy produces hepatotoxicity is poorly understood and is thought to involve hypersensitivity or immunologic reactions in addition to the direct cytotoxic effects of its metabolites.

Hepatotoxicity was evident as shown by an increase in liver enzymes, ALT, AST and ALP and a decrease in total protein levels. Photomicrographs of the liver of test rats, that received high dose of disulfiram/copper gluconate combination, for 45, 60 and 90 days, showed generalised severe cytoplasmic vacuolation.

Lipid profile indices recorded an increase in triglyceride levels and total cholesterol levels. A decrease in HDL-C was also observed.

CONCLUSION

From the results obtained, the disulfiram/copper gluconate combination showed significant ($P < 0.05$) elevation in AST, ALT and ALP indicative of liver damage. The atherogenic index which showed a high level of triglyceride to a decreased level of HDL-C may imply increased tendency for the development of atherosclerosis. At high doses this therapeutic agent is toxic and should therefore be used with caution.

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