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Spring Time and Autumn Time Toxic Effects of Copper (II), 3-(2-Furyl) Prop-2-Enal Semicarbazone and $[\text{CuCl}_2(\text{FASC})_2]$ Complex in Mice

B. Jamali¹, G. Ibrahim², G. Bouet², M.A. Khan², P. Allain¹ and X. Do Thanh^{1*}

ABSTRACT

In vitro, 3-(2-furyl) prop-2-enal semicarbazone-copper (II) complex $[\text{CuCl}_2(\text{FASC})_2]$ presents anti-mitotic effects. In this work we studied the *in vivo* seasonal toxic effects in male Swiss mice of CuCl_2 , and FASC and the $[\text{CuCl}_2(\text{FASC})_2]$ complex. In spring, one injection of CuCl_2 $8 \cdot 10^{-2}$ mmol killed 16% of animals after 24 h. Cupric chloride lethal dose was up to $64 \cdot 10^{-2}$ mmol with 100% mice dead after 24 h. FASC was well tolerated from 0.65 to 1.3 mmol. The complex was 100% lethal with $48 \cdot 10^{-2}$ mmol. In autumn, mice were more sensitive to CuCl_2 and to the complex with lethal doses up to $32 \cdot 10^{-2}$ mmol and $8 \cdot 10^{-2}$ mmol, respectively. On the other hand, FASC was well tolerated. It is concluded that the *in vivo* toxic effects of CuCl_2 and $[\text{CuCl}_2(\text{FASC})_2]$ complex are quite different in spring and autumn.

Abstracting keywords: Mice, toxic effects, CuCl_2 , FASC, $[\text{CuCl}_2(\text{FASC})_2]$.

INTRODUCTION

Usually *in vivo* studies of molecule effects do not take into account rhythmic changes in internal biological susceptibility to chemical agents or drugs, as well as the ethology of experimental subjects. Mice and rats are rodents that are very active during seasons with days longer than nights (spring and summer) and rest in autumn and winter with days shorter than night (Laroche & Rousselet, 1990). Many authors such as Reinberg et al. (1991), Cambar et al. (1994), Bruguerolle (1994), Touitou and Haus (1994) related experiments on rodents and human beings on the subject of the moment of administration (hour of the day, day of the month, month of the year) of drugs and their toxic effects. Cambar et al. (1994), showed that the same dose of mercuric chloride in intraperitoneal (i.p.) injection induced a different mortality rate when given in November and in February. In

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rats, a metabolite of vitamin D₃ was more active during night time than day time on renal enzymatic markers (Do Thanh et al., 1994) which presented annual variations (Do Thanh et al., 1995). Effects of cobalt (II), 1 α ,25-dihydroxyvitamin D₃ and [CoCl₂(1,25(OH)₂D₃)] complex were quite different in spring and in autumn (Do Thanh et al., 1996).

Our laboratory focused on studying chemical and/or physiological effects of metals and semicarbazones. 3-(2-furyl) prop-2-enal semicarbazone (FASC) is an anti-tumoral (Blanz & French, 1968), anti-inflammatory (Hall et al., 1996) and anti-fungal agent (West et al., 1988). It is reported that cupric chloride is the main cofactor of many enzymatic systems. Copper (II) is an anti-inflammatory metal and would have some effects in cancerogenesis by protecting from free-radicals effects (Hall et al., 1996).

Unfortunately CuCl₂ itself is a dangerous product. It is well known that most molecules lose or change their physiological effects when complexed. Our laboratory has synthesized complexes between CuCl₂ and FASC and the most stable obtained was [CuCl₂(FASC)₂]. Preliminary *in vitro* studies revealed that this complex presented antimitotic effects (Hall et al., 1996). It is therefore interesting to study its toxic effects in mice, in spring and autumn before further *in vivo* experiments, as knowledge of these administration times can coincide with best tolerance or effectiveness. These results are necessary in order to optimize both timings and dosages of any anti-mitotic molecule.

MATERIAL AND METHODS

Preparation of the compounds

Cupric chloride CuCl₂, 2 H₂O (Prolabo p.a.) is dissolved in the vehicle. FASC, whose structure is given (Fig. 1) and the complex [CuCl₂(FASC)₂] were prepared as previously described (Ibrahim et al., 1997) and dissolved in propan-1,2-diol.

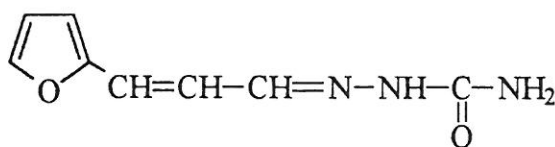


Fig. 1. Structure of 3-(2-furyl) prop-2-enal semicarbazone (FASC).

Animals used

Male animals used were Swiss mice from DEBRE (France) weighing from 28 to 34 g. Male mice were chosen because of their regular weight gain and the absence of oestral cycles with changes in sexual hormonal status which could cause some errors in *in vivo* experiments. Animals had free access to both water and standard

diet containing 0.8% Ca, 0.7% P and 3 IU/g Vitamin D. They were placed at room temperature $20 \pm 2^\circ\text{C}$, and exposed to day light.

Experiments with CuCl_2

In addition of the control series, eight series of twelve mice were used and each animal received a single intraperitoneally injection ($200 \mu\text{l}/30 \text{ g}$ body weight: b.w.) between 10 O'clock and 12 h. On the 10th of May, rodents were treated as follows :

- * control series : animals received the vehicle (propane-diol)
- * series 1 : 10^{-2} mmol
- * series 2 : $2 \cdot 10^{-2} \text{ mmol}$
- * series 3 : $4 \cdot 10^{-2} \text{ mmol}$
- * series 4 : $8 \cdot 10^{-2} \text{ mmol}$
- * series 5 : $16 \cdot 10^{-2} \text{ mmol}$
- * series 6 : $32 \cdot 10^{-2} \text{ mmol}$
- * series 7 : $64 \cdot 10^{-2} \text{ mmol}$

All animals were placed again at room temperature and day light during 6 days and the same protocol was used in the autumn (10th of November).

Experiments with FASC

Two series of twelve mice, in addition to the control series, were used and each animal received a single i.p. injection ($200 \mu\text{l}$ per 30 g b.w.) between 10 and 12 h in the morning. On the 10th of May, rodents were treated as follows :

- * control series : animals received the vehicle (propane-diol)
- * series 1 : 0.65 mmol
- * series 2 : 1.3 mmol

The same protocol was used in the autumn (10th of November).

Experiments with $[\text{CuCl}_2(\text{FASC})_2]$

Seven series of twelve mice and the control series were used and each animal received a single i.p. injection ($200 \mu\text{l}/30 \text{ g}$ b.w.) in the morning from 10 to 12 h. On the 10th of May, rodents were treated as follows :

- * Control series : animals received the vehicle (propane-diol)
- * series 1 : $2 \cdot 10^{-2} \text{ mmol}$
- * series 2 : $4 \cdot 10^{-2} \text{ mmol}$
- * series 3 : $8 \cdot 10^{-2} \text{ mmol}$
- * series 4 : $16 \cdot 10^{-2} \text{ mmol}$
- * series 5 : $32 \cdot 10^{-2} \text{ mmol}$
- * series 6 : $48 \cdot 10^{-2} \text{ mmol}$
- * series 7 : $64 \cdot 10^{-2} \text{ mmol}$

The same protocol was used in the autumn (10th of November).

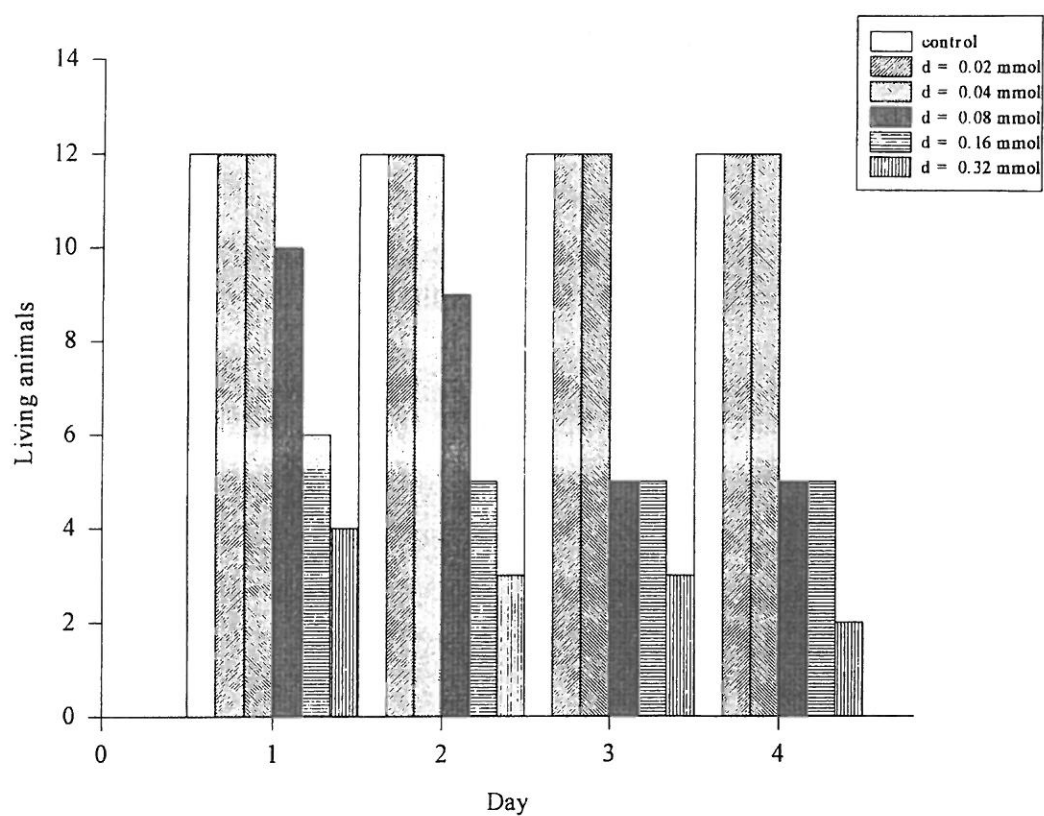


Fig. 2. Toxic effects of CuCl_2 in Spring. All animals were killed the first day in the series 7.

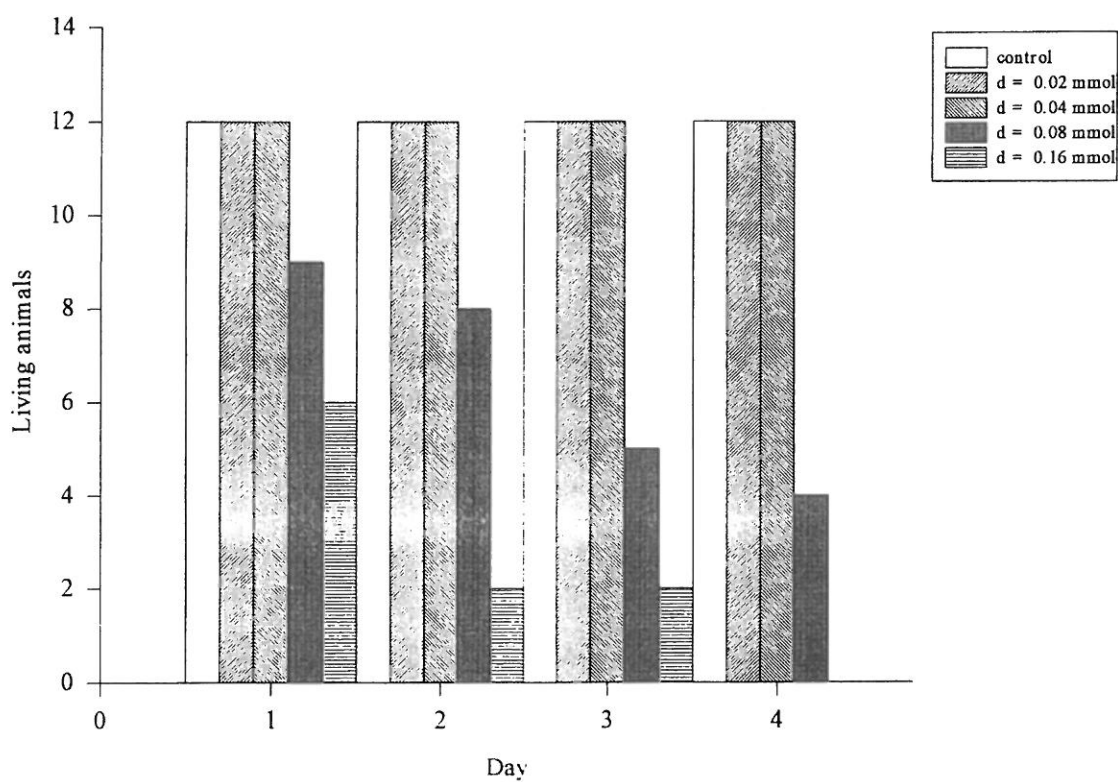


Fig. 3. Toxic effects of CuCl_2 in Autumn. All animals were killed the first day in the series 6 and 7.

RESULTS

In spring time, 24 h after i.p. injection, cupric chloride from 10^{-2} mmol to $4 \cdot 10^{-2}$ mmol did not apparently harm the mice under study (Fig. 2). But $8 \cdot 10^{-2}$ mmol killed 16% (2/12) after 24 h; then from 48 to 96 h two more mice died (33%). A dose up to $64 \cdot 10^{-2}$ mmol was lethal (100%) after 24 h (Fig. 2).

In autumn, copper (II) chloride was more effective. Lethal dose (100%) was up to $32 \cdot 10^{-2}$ mmol. These results proved that CuCl_2 was toxic to mice, and more toxic in autumn than in spring (Fig. 3).

FASC was well tolerated by rodents in spring and in autumn with respect to our experimental conditions.

In spring, the complex had no effect in mice from $2 \cdot 10^{-2}$ mmol to $4 \cdot 10^{-2}$ mmol. A dose up to $8 \cdot 10^{-2}$ mmol killed 58% animals 96 h after injection. A higher effect (75%) was obtained after 24 h when the dose was doubled. Lethal dose (100%) was up to $48 \cdot 10^{-2}$ mmol (Figs. 4 and 5).

In autumn, complex toxicity was obtained with only $8 \cdot 10^{-2}$ mmol (100%) 24 h after administration : so mice were more sensitive to the complex in autumn than in spring.

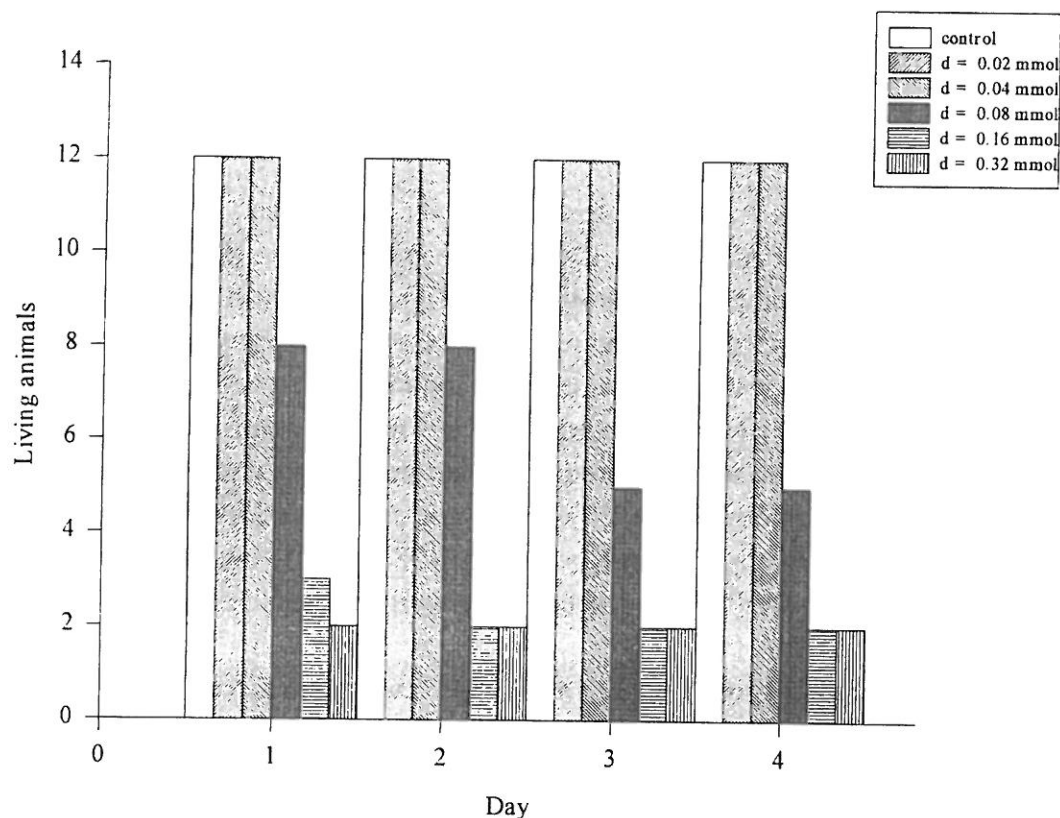


Fig. 4. Toxic effects of the complex $[\text{CuCl}_2(\text{FASC})_2]$ in Spring. All animals were killed the first day in the series 6 and 7.

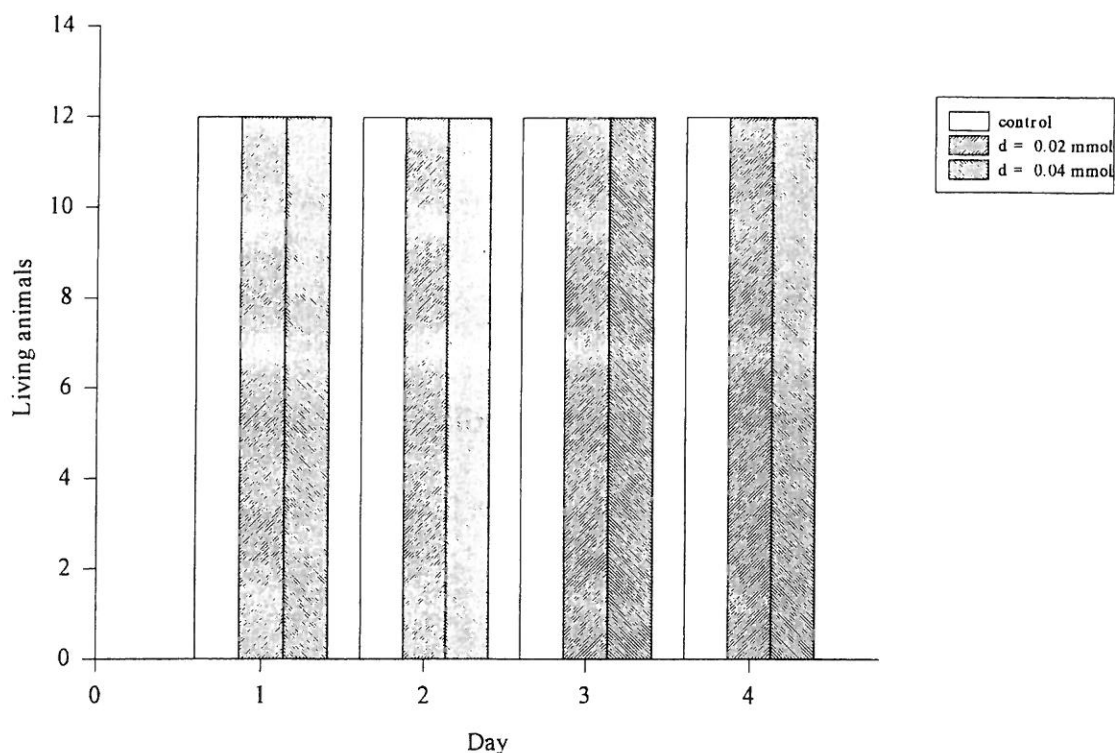


Fig. 5. Toxic effects of the complex $[\text{CuCl}_2(\text{FASC})_2]$ in Autumn. All animals were killed the first day in the series 3 to 7.

DISCUSSION

This work proved that in mice, tolerances of the CuCl_2 , and 3-(2-furyl) prop-2 enal semicarbazone (FASC) and $[\text{CuCl}_2(\text{FASC})_2]$ complex were quantitatively different in May as compared to November (Figs. 2, 3, 4 and 5) in rodents kept under daylight during two different seasons of the year : spring and autumn.

Many experiments were undertaken showing circadian chronestesy and circadian toxic effects of different molecules. For example in mankind, corticoids are not well tolerated when administered in the afternoon but well supported in the morning (Gervais & Reinberg, 1987). The same considerations could be applied to adriamycin (Hrushesky & Von Roemeling, 1986), while cis diammine dichloroplatinum (II) (Cis-DDP) is on the contrary well tolerated in the late afternoon (18.00 h).

However, according to Cambar et al. (1994) few papers showed seasonal chronoeffects of drugs. This may be due to the magnitude and the cost of such experiments. Our toxic effects studies, dealing with the periodic changes of activity of CuCl_2 , FASC and $[\text{CuCl}_2(\text{FASC})_2]$ complex in living mice, could be correlated with previous works. According to Levi (1987) tolerance of adriamycin in rodents was maximal in autumn and less in spring. Bruguerolle et al. (1988), demonstrat-

ed that the acute toxicity of phenobarbital in mice varies during the year long: the barbiturate is more toxic in January and is well supported in July.

In our experiments, mice were kept under constant temperature. According to Reinberg et al. (1991) experiments under constant conditions (for example temperature) with laboratory animals during many seasons showed that their seasonal rhythms were not altered. But our mice were kept also under daylight in spring and in autumn. Our experimental conditions were correlated with studies made by Tosini and Menaker (1996). These authors demonstrated that there are circadian rhythms in cultured mammalian retina under constant temperature and different light periods. Our results are in harmony with these previous studies. When CuCl_2 , FASC and the complex were injected in spring, mice tolerancy to CuCl_2 from 10^{-2} to $4 \cdot 10^{-2}$ mmol was better and was progressive. Cupric chloride became strongly toxic to rodents with $16 \cdot 10^{-2}$ mmol. The complex was well tolerated from $2 \cdot 10^{-2}$ to $4 \cdot 10^{-2}$ mmol, but killed 75% animals 24 h after injection with a dose up to $16 \cdot 10^{-2}$ mmol. FASC did not harm the mice under study. It is interesting to note that when it is complexed with FASC, CuCl_2 presented a lower toxicity to mice.

In autumn cupric chloride and complex toxicities were rapid and abrupt and lethal (100%) doses were of very low concentration.

We can explain the mice seasonal toxic effects to CuCl_2 and to the complex on the basis of seasonal variations. All living beings present biological variations so they can react differently to the same drugs when injected during various seasons in the same year (Cambar et al., 1987). This has been explicitly shown in the case of other molecules by variations of their kidneys and brain tolerances (Cambar et al., 1987; Dorian et al., 1987; Mato et al., 1981; Do Thanh et al., 1996).

For further experiments, we will have to take in account the importance of the seasonal changes, as well as the circadian variations of tolerances towards toxic molecules. In conclusion, we postulate that (i) mice toxic effects to drugs is related to mammalian seasonal biovariability; (ii) it could be influenced directly by light cycles and (iii) copper (II) is to be complexed in order to lower its own toxicity.

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