Toxicological Tests of Tumolit and Artemisinin Products on Laboratory Mice and Rats

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Abstract

This paper presents the experimental results establishing the lethal dose (LD) of the two products considered to have antitumor effect: Tumolit and Artemisinin.

For testing, we used an experimental design including two species of gnawers, Swiss mice and Wistar rats. Two experimental groups were used, consisting of 20 Swiss male mice each, as well as two groups of 10 Wistar rats/lot. In interpreting the results of the research and in the development of the experimental protocol, we took into account the toxicity scale after Hodge and Sterner. Initial doses were used of both products at 200mg / kg / body for each mouse and rat, respectively, administered orally in single-doses. Between each oral dose, there was one day of rest in order to evaluate possible effects. As mentioned in the Protocol, the dose was increased to 500mg dose / kg / body, then to 1000 mg / kg / body, 2000mg / kg / body and then to 5000 mg / kg / body. At this last dose, administration was disrupted in mice. For rats, doses were further increased in three innings to 5000 mg / kg / body, 15,000 mg / kg / body concerned 20,000 mg / kg / body in single oral dose. The administration was done by gavage in a single dose. There were no toxicity effects reported at any dose level applied, which is why we conclude that the two products are practically non-toxic.

Keywords: *Artemisinin, mice, rats, tests, toxicity, Tumolit.*

INTRODUCTION

In this paper, we present the experimental results on the median lethal dose (LD₅₀) of the two products considered to have antitumor effect: Tumolit and Artemisinin (producers 'prospect-Parapharm, Cluj; Houet *et al.*, 2008; Lai *et al.*, 2013, Das, 2015).

Artemisinin is the active substance in the plant *Artemisia annua*, known as sweet wormwood (Efferth and Kaina, 2010). From a chemical point of view, Artemisinin is a sesquiterpene lactone with an endoperoxide bridge composed of three isoprene units and a lactone ring (www.ebi.ac.uk).

Tumolit, a dietary supplement considered by producer (Parapharm Cluj) to have antitumor effect, has the following composition: arginine, vitamin C, L-phenylalanine, L-histidine, D,

L-methionine, malic acid, L-tryptophan, adenine, L-tyrosine, vitamin B6, vitamin B2, biotin, iron, zinc, magnesium, manganese, copper, molybdenum, vanadium, nickel, boron, fluorine, cobalt (according to information provided by the producer - Parapharm Cluj).

The lethal dose (LD) indicates an acute toxicity of substances that enter the body orally or parenterally, representing the minimum dose of the substance that causes the death of an adult animal. The lethal dose is established on an animal experimental batch and marked with DL followed by an index - from 1 to 100- indicating the percentage of lethality, at a given time; the expression is in mg / kg body weight (Gofita, 2011).

The variation in individual response to lethal doses of a substance required input values (Danila *et al.*, 1984): DL_5 (DLM) = minimum lethal dose, LD_{50} (DML) = median lethal dose, DL_{75} = fatal dose, DL_{100} = maximum lethal dose (absolute lethal dose), DL_0 = maximum tolerated dose (with toxic effects but not fatal), often used in practice, it is not actually a lethal dose.

Typically, in order to express the acute toxicity of a substance, the LD_{50} category is used (mg / kg), because at this dose, individual reactivity differences are lower. This is a virtual value, obtained by statistical calculation, not a biological constant. Many factors can influence the toxicity, thus affecting the expression LD_{50} . Such factors include animal breed, age and weight, the nature of food, environmental conditions, absence of feeding before performing the test, the method of administration, the volume and nature of conditioning the environment, duration of animal observation. Because of these variables, for many purposes it is sufficient only to determine the order of magnitude for LD_{50} (eg. between 5 and 50 mg / kg body weight and 500 mg / kg, etc.), not the absolute value (Gofita, 2011). In relation to LD_{50} we can classify and compare toxic substances.

Lethal dose 50 is usually determined on laboratory animals such as mice, rats, rabbits, guinea pigs. Although other tests are under development, which may, to some extent, replace the current form test, classical measures are still among the most important in measuring toxicity. Many events led to the implementation and further use of these tests, although the test population is significantly reduced compared to their original size (Encyclopaedia of Toxicology, 2014). Result evaluation for our research was done according to Hodge and Sterner toxicity scale shown below (tab.1).

MATERIALS AND METHODS

In practice, a lethal dose is recognized according to three coordinates: - minimum- DL_{25} , - medium - DL_{50} and maximum- DL_{100} . In our research, we tried to establish LD_{50} , which represents the dose at which death occurs in 50% of experimental animals.

The purpose of these measurements was to establish a possibly toxic or even lethal effect of the two products administered, Tumolit or Artemisinin, a dose-dependent effect. Knowing that toxicity depends on exposure to the toxic compound, and this is, in turn, determined by the dose and duration of exposure, we tried to assess this risk by establishing LD_{50} and monitoring all parameters that could, in some way, influence the average lethal dose determination.

The material studied was Tumolit, a dietary supplement, and Artemisinin, regarded as having antitumor effect (Golenser *et al.*, 2006).

For each type of supplement tested, there was one experimental group consisting of 20 mice of a Swiss variety and 10 Wistar male rats. The experimental groups were kept under identical conditions of temperature ($22^{\circ} \pm 0.5^{\circ} \text{C}$) program of 12/12 hour light-dark, ad libitum feeding fodder, a granulated product provided by the Cantacuzino Institute, which has the following composition: corn, soybean meal, sunflower meal, calcium carbonate, calcium phosphate, amino acids, salt, vitamin-mineral premix and water ad libitum. Tests were conducted in the Quantum Pharm company laboratory, Cluj-Napoca.

As a novelty, in our research we used a toxicity test progressively, from low doses to high doses. The advantage of this method is that it reduces the number of units required for experimental testing, avoids losses, duplication of testing on other individuals and groups.

Tab.1. Hodge and Sterner Toxicity Scale- Group- Toxicity Degree DL_{50} - single oral dose/rat

Toxicity Group	Toxicity Degree	DL_{50} – single oral dose/rat
1	extremely toxic	under 1 mg /kg
2	very toxic	1 - 50 mg /kg
3	moderately toxic	50 - 500 mg /kg
4	low toxicity	500 - 5 000 mg /kg
5	practically nontoxic	5 000 - 15 000 mg /kg
6	relatively nontoxic	over 15 000 mg /kg

The doses used were administered with a 48-hour difference, considering that their effect will fade in this time. The experiment lasted nine days on mice and 11 days on rats. Tumolit administration, as well as the product Artemisinin doses / mice / rats as shown in Table 2.3 was made orally by esophageal gavage, the product was mixed with distilled water in the amount of 0.5 ml / mouse and 1 ml / rat. The doses per day were: (tab.2) (Table 3)

During the test, the behaviour of individual groups and the experimental units was observed, in terms of clinical signs of intoxication and body weight by weighing.

RESULTS AND DISCUSSION

At an amount of 6 mg/mouse or 28 mg/rat in both Tumolit and Artemisinin, there were no reported changes in group or individual behaviour or appetite.

The same observations resulted at a dose of 30 mg/mouse and 140 mg/rats, administered on day five and recorded until the seventh day, whereas Artemisinin, administered at 60 mg /mouse which corresponds to 2000mg / kg / body, and 280 mg

/ rat, in both species led to a tremor manifested by continuous motion in the first two hours following administration. There were no other events typical of a chemical poisoning, whereas the appetite was normal, although we found a slight reduction in weight for all individuals. The signs of agitation disappeared in the first 24 hours after administration, and in the next 24 hours, the behaviour returned to normal. The same signs and behaviours can be reported in Artemisinin to an amount of 150 mg/700 mg for mice/rats, respective. Tumolit did not lead to behavioural changes or weight loss. There were no clinical signs of intoxication with any of the products administered.

During the 11 days of the experiment there was no mortality. After testing, both groups of mice and rats were observed for 14 days to determine if any signs of late intoxication could occur. During this period the same type of feed was administered, we used the same conditions of light, temperature and water at discretion. In this interval, follow-up post-test animals exhibited normal behaviour without any clinical changes.

Tab. 2. The doses of the product Tumolit used in the experiments

Day	Dose mg/kg/body	Dose/Mice Swiss(mg)	Dose/Rats Wistar(mg)	Obs.
1.	200 mg	6 mg	28 mg	Without clinical signs
3.	500 mg	15 mg	70 mg	Without clinical signs
5.	1000 mg	30 mg	140 mg	Without clinical signs
7.	2000 mg	60 mg	280 mg	Without clinical signs
9.	5000 mg	150 mg	700 mg	Without clinical signs
11.	10,000 mg	-	1400 mg	Without clinical signs

Tab. 3. The doses of the product Artemisinin used in the experiments

Day	Dose mg/kg/body	Dose/Mice Swiss(mg)	Dose/Rats Wistar(mg)	Obs.
1.	200 mg	6 mg	28 mg	Without clinical signs
3.	500 mg	15 mg	70 mg	Without clinical signs
5.	1000 mg	30 mg	140 mg	Without clinical signs
7.	2000 mg	60 mg	280 mg	Agitation, without clinical signs, weight loss
9.	5000 mg	150 mg	700 mg	Agitation, without clinical signs, weight loss
11.	10000 mg	-	1400 mg	Agitation, without clinical signs, weight loss

CONCLUSIONS

1. The Tumolit product in the aforementioned dose did not produce any change in the experimental units.
2. The Artemisinin product determined, at the starting dose of 2000 mg / kg / body, mild manifestations of agitation, stagnation or slight reduction in body weight without any specific signs of intoxication.
3. We conclude that neither Tumolit, nor Artemisinin produce toxicity at a dose of 500 mg / kg / body, which allows us to state that they present no toxicity.

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