



ANALYSIS OF HEMOGLOBIN (HB) CONCENTRATION IN CIRCULATING BLOOD OF MICE AFTER INTRA-PERITONEAL INJECTION OF ISCADOR

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ABSTRACT

The aim of this study was the analysis of the use of Iscador Q as a potential anti-carcinogenic and immunomodulatory drug that affects the concentration of hemoglobin (HB) in the peripheral blood of mice. Iscador Q was injected in the doses of 1, 3 and 5 mg/kg of body mass. The concentration of hemoglobin was marked by Drabkin's Method. The highest concentration of hemoglobin was observed after the highest dose of Iscador Q was injected. All results were statistically significant.

Key words: hemoglobin, Iscador Q, mice, viscumins, viscotoxins

INTRODUCTION

Hemoglobin is protein essential to gas exchange. Any fluctuations in hemoglobin level in blood have significant influence on the metabolic performance and state of health of human or animals (Dąbrowski,1998). Biosynthesis of hemoglobin is strictly controlled by the organism, although numerous health disorders as well as feeding, toxins and physical factors can significantly influence hemoglobin concentration and formation of erythrocytes (Powell et al., 1982; Shih, et al., 2007). Proper hemoglobin concentration in blood is especially

important in patients with chronic diseases. This is why so much attention is paid to proper nutrition and possible application of diet ingredients during chronic illness.

Within the last few years plant anti-carcinogenic products are taken under consideration by scientists. One of them is Iscador, acquired from mistletoe *Viscum album* L. (**Kuttan et al., 1990**). (IsCADOR has wide influence on biological systems, and affects activity of leukocytes system. IsCADOR contains lectins, which act as stimulators of immune system. Medicines containing IsCADOR increase erythropoiesis processes in bone marrow. The aim of this study was to determine effects of different doses of IsCADOR on iron concentration in blood serum of mice (**Harmsma, et al., 2004**).

MATERIAL AND METHODS

The experiment was carried out on 24 male mice of Swiss strain, average body weight 25 to 26g, fed standard diet with unlimited access to water. All animals were divided into four groups: control-one and three experimental groups, six individuals in each of them. Mice from the control group were administered 0.3 ml of physiological saline as an intraperitoneal injection, whereas the mice of the first experimental group were given 1 mg/kg of body mass of IsCADOR Q, mice of the second group were injected with IsCADOR Q in the dose of 3mg/kg of body mass, and the last group was given 5mg of IsCADOR Q per kg of body mass. 24 hours after the injection animals were decapitated and blood samples were collected.

Concentration of hemoglobin was marked by Drabkin's Method, spectrophotometrically at 540 nm.

Blood samples (0.02 ml) were taken from the carotid artery and added to 5 ml of Drabkin reagent containing: 0.048 g / l KCN, 0.18 g / l K Fe CN, 0.136 g / l KHPO and 0.1 g / l of detergent. Next the samples were mixed and incubated at room temperature for 5 min.

The reagents were stored at 4-8 ° C. For the measuring a standard solution of 16,3 g/dl hemoglobin concentration and distilled water was also used.

The results were analyzed using Student's test t. The statistical differences were defined at $p < 0.05$.

Measuring hemoglobin with the cyanmethemoglobin method (Drabkin's Method)

- Random sample: 5000 µl of Drabkin reagent + 20 µl of distilled water
- Standard: 5000 µl of Drabkin reagent + 20 µl of sample solution of hemoglobin

- Target sample: 5000 µl of Drabkin reagent + 20 µl of capillary blood

The concentration of hemoglobin was marked with Drabkin's method, with the use of SPEKOL 11 spectrophotometer, at 540nm wavelength. 10 mm optical path length quartz spectrophotometric cuvettes were employed in the testing. Once Drabkin reagent was mixed with capillary blood the solution was incubated at room temperature for the duration of 5 mins and absorbancy was measured. The spectrophotometer was set to zero using distilled water.

Measuring formula

Hemoglobin and its derivatives affected by Drabkin reagent transform into cyanmethemoglobin, whose optical density may be measured photometrically.

The concentration of hemoglobin was calculated according to the following formula:

$$\text{Hb (g/dl)} = \frac{A_T}{A_S} \times c_w$$

where:

A_T - absorbancy of tested sample

A_S - absorbancy of standard

c_w - concentration of standard in g/dl

RESULTS AND DISCUSSION

Statistical analysis of the results showed significant increase in hemoglobin concentration in peripheral blood of mice.

The average concentration of hemoglobin in the control group was 13.77g / dl, while in the experimental group, which was given a dose of Iscador Q of 1mg/kg by the average concentration of hemoglobin was 17.13 g / dl. Mice of the second experimental had mean hemoglobin concentration of 17.67032 g / dl. Finally mean hemoglobin concentration in animals of the last group was 20.33 g / dl. The results shown in Table 1.

After intra-peritoneal injection of Iscador Q in the dose of 1 mg/kg of body mass the concentration of hemoglobin rose by 124,39%. After the application of Iscador Q in the dose of 3 mg/kg of body mass the concentration of hemoglobin rose by 128,27%, while after the application of Iscador Q in the dose of 5 mg/kg of body mass the concentration of hemoglobin rose by 147,64%.

Where at all the results were statistically significant at $p < 0,001$.

The results of this experiment showed that hemoglobin concentration was positively related to the doses of Iscador.

Table 1 Hemoglobin (Hb) concentration in male mice after different doses of Iscador Q

group	N	Hb in g/dl	Hb in %	SD	Test „t”
Control group	6	13,78	100	0,045	-
Group 1	6	17,14	124,39	0,027	155,09***
Group 2	6	17,67	128,27	0,032	170,71***
Group 3	6	20,34	147,64	0,032	282,22***

*** the differences were statistically significant at $p < 0,001$ in comparison to control

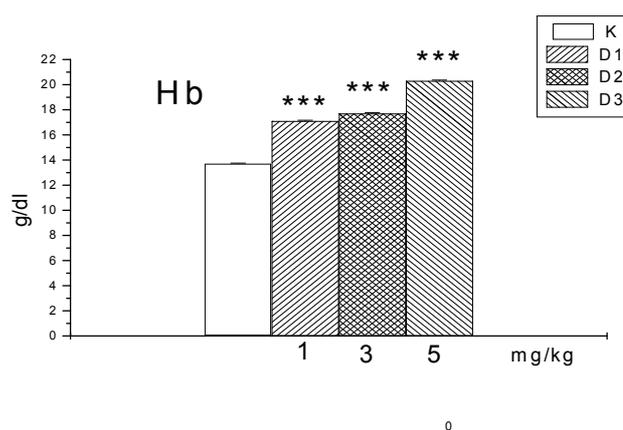


Figure 1 Hemoglobin concentrations (g/dl) in the peripheral blood of mouse after application of Iscador Q (*** the differences were statistically significant at $p < 0,001$)

Our results indicate that substances present in Iscador Q have positive influence on hemoglobin concentration in blood of mouse. Other authors also indicated that Iscador increases blood hemoglobin (**Jurin et al.,1993**). The Iscador influence on hemoglobinsynthesis was probably related to the presence viscumin (lectins) and viscotoxin and flavonoid compounds (**Jurin et al., 1993**).

Lectins present in Iscador Q such as ML I, ML II, ML III are structural components of membranes and cell walls, many of them show enzymatic activity. They are characterized by the ability of agglutination of viruses, bacteria and erythrocytes, and therefore were named as fitoaglutynins (**Kuttan and Kuttan, 1993**).

Viscumins are glycosylated cytotoxic proteins particle weight of 56-64 kDa, showing varied specificity of binding to carbohydrates. Viscumin ML I is primarily *D-galactose-specific*, while ML II and ML III show specificity of binding to N-acetyl-D-galactosamine (**Bussing, Schietzel,2007**). The above-mentioned viscumins are probably the products of one gene, with the resulting isoforms being the effect of post-translational modifications (generated either in the plant itself or in the drug manufacturing process) and differ only in their particle weight, which results from their differing glycosylation degree (**Janssen, Scheffler, Kabelitz, 1993**)

The primary objective of the study is viscumin ML I that, similarly to other viscumins, consists of catalytically active subunit A showing the RNA N-glycosidase activity and subunit B working as lectin (**Harmsma et al.,2004**)

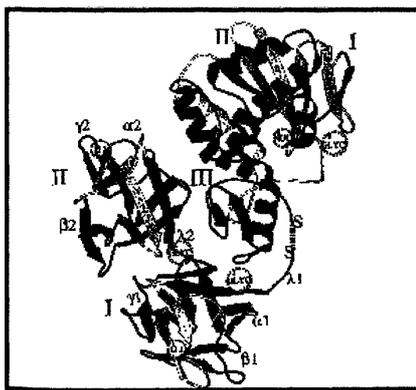


Figure 2 Ribbon model of viscumin MLI according to Krauspenhaar et al. (1999)

Viscotoxins are small basic proteins rich in cysteine residues which belong to the family thionins. Thionins show the greatest toxicity to bacterial cells, fungal cells, plants and animals. There are 6 isoforms of viscotoxins, among which may be distinguished: A1, A2, A3, B, 1-PS, U-PS. Among the viscotoxins isolated from mistletoe extracts viscotoxin A3 is considered to be the most biologically active, thus it makes the primary objective of research.

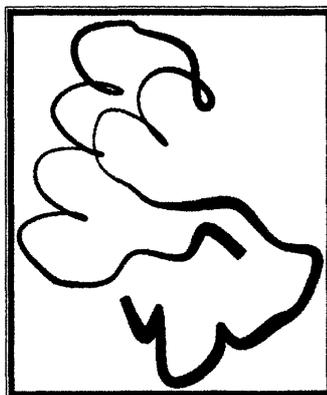


Figure 3 Viscotoxin A3 particle structure (Goerter, 2003)

An extraintestinal application of viscotoxins produces a negative inotropic effect, causing bradycardia, lowering heart rate and lowering blood pressure. Tissue necrosis develops at the injection site, thus viscotoxins may be used to destroy skin or sub-skin tumors (Rózański, 2003).

Among other chemical constituents of mistletoe may be found the following:

- Biogenic amines: histamine, tyramine, *phenylalamine*, tryptamine,
- Amino acids: gamma-Aminobutyric acid, valine, leucin, arginin,
- terpene compounds: oleanolic acid, ursolic acid, beta-Sitosterol,
- Mucilages and alkaloids
- Flavonoids (e.g. quercetin) and carotenoids (e.g. xanthophyll),
- Phenols: caffeic acid, ferulic acid, sinapinic acid, vanillic acid (Rózański, 2003).

Viscumins and viscotoxins present in Iscador Q show also an inhibiting influence on viral infections. They contribute to the reduction of reactive oxygen species resulting from reactions catalyzed by iron ions. These compounds are cytotoxic to cancer cells. Because of binding to sugar residues of membrane proteins they have the ability to aggregate cancer and normal cells in the absence of toxicity towards them (Kaegi, 1998; Olaku and White, 2011)

Flavonoids have strong antioxidant properties, showing the ability to scavenge free radicals and chelation of heavy metals in the body. Their antioxidant activity is related to the

inhibition of oxidation of endogenous antioxidants such as ascorbic acid (vitamin C), glutathione and tocopherol (vitamin E). A significant role is played by flavonoids such as quercetin and rutin which are antioxidants in relation to vitamin C, blocking the conversion of ascorbic acid to dehydroxyascorbic acid, protecting against free radicals. By contrast, ascorbic acid delays the oxidative metabolism of flavonoids and thus extend their protective effect. Flavonoids have an antioxidative properties. These properties are very important to the removal of reactive oxygen species from blood and prevention against the formation of oxygen free radicals (**Olaku and White, 2011**). The mechanism of this action is related to the inhibition of enzymes such as lipooxygenase, cyclooxygenase, and xanthine oxidase, associated with the generation of free radicals (**Janssen et al., 1993**). Iscador influence on the process of cell proliferation and an increase in hemoglobin concentration in peripheral blood of mice is closely related to hemopoiesis. Other studies have shown significant increase in blood iron level (**Żala, 2008**) and hemoglobin concentration after Iscador Q administration (**Olaku and White, 2011**).

CONCLUSION

1. Administration of Iscador Q leads to significant increase in hemoglobin concentration in peripheral blood of mice compared to control group
2. The increase in hemoglobin is directly proportional to the dose of Iscador Q
3. It seems that a significant increase in hemoglobin concentration in peripheral blood of mice is closely associated with the process of hemopoiesis, which is stimulated by the Iscador, especially viscumins and viscotoxins.

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