

HAEMOLYTIC ACTIVITY OF RHISOME AND ROOT EXTRACT OF *Helleborus odorus* WALDST. ET KIT. APPLIED ON WISTAR RATS **

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** Original scientific paper

Abstract: In the health care of animals phytoteraphy is being applied very intensively, mostly for the prophylactic purpose and with the aim of treating weaker forms of diseases and chronic course of a disease. It is well known from a traditional folk medicine that *Helleborus* L. can induce the functions of defensive cell effectors and increase the activity of rapid, unspecific mechanisms of immune response. The plants of the genus *Helleborus* L. contain saponosides which increase the permeability of the erythrocytes membrane and that leads to the haemolysis and loss of haemoglobin.

In this research paper we have studied the influence which the rhisome and root extract of *H. odorus* Waldst. et Kit., may have on the value of the erythrocytes count, concentration of haemoglobin and haematocrit value. In the rats in the trial group the extract of underground plant organs of *Helleborus odorus* Walds. et Kit. was applied intramuscularly in the concentration of 0,5, 5 or 20mg/100g TM. In the rats in a control group a sterile physiological solution in the quantity of 0,25ml/100g TM was applied in the same way.

Key words: *H. odorus* Waldst. et Kit., haemolytic activity, rats

Introduction

Medicinal herbs, along with some other herbs, are used both separately or as an additional therapy to conventional drugs which can, in this case, thanks to the action of active plant ingredients, be used in lower, safer doses. Because of an irritable effect on the skin and mucuous membrane, there is a

custom for the rhizom of *Helleborus* L. to be used for transcutaneous implantation, to protect animals from diseases and parasites. This natural method of treatment is used especially in Pek, Zvižd and Jermenovci (Đurić, 1985), in Vojvodina (Tucakov, 1996), Romania (Bogdan et al., 1990) and Macedonia (Tosevski et al., 2004). Bogdan et al. (1990) have confirmed a clearly indicated leukocytosis and neutrophilia, lasting over 6 days, in all farm animals which had the rhizom of *H. purpurascens* W. et K. implanted transcutaneously. The results of haematologic analysis and the change in body temperature in rats treated by different concentrations of the extract of underground organs of *H. odorus* Walds. et Kit. have indicated the increased activity of rapid, unspecific defensive mechanisms (Milanović et al., 2004; Davidović et al., 2006). A purified extract of *Helleborus* L. can also cause some modifications in immune response in the state of primary or secondary immune deficiency (Bolte et al., 2001).

A great number of saponoside drugs show an immunostimulative action on the organism, can induce its functions and display antibiotic, antimycotic and antiviral activity. Saponins have a very strong irritative effect, inducing the contractions of rumen and accelerating the motility of intestines and uterus, for which reason the *Helleborus* L. was used as a purgative, local contraceptive and for inducing the abortions (Bogdan et al., 1990). The aglycaemian components of steroid saponosides have been isolated from some of the species of genus *Helleborus* L., and their structure established, at which a conclusion was reached that some species of hellebore do not contain the same saponins (Colombo et al., 1990).

Biological and pharmacological effects of saponosides are based on their characteristic that they represent a surface active compounds, increasing the permeability of erythrocytes membrane what leads to haemolysis and loss of haemoglobin. Studying the haemolytical effect, Petričić et al. (1971) proved the presence of saponosides in the underground plant parts of all examined species of the genus *Helleborus* L. and confirmed that haemolytical activity was not in the correlation with their quantity, but that it depends on their structure. The most intensive haemolysis is being induced by saponins having one carbohydrates chain containing 4-5 monosaccharides, while with the elongation of sugar chain a haemolytical activity is being decreased. Haemolysis is also influenced by a branching of carbohydrate chain. By a comparative research of haemolytical activity of saponosides these authors have confirmed a great difference not only among certain species of the genus *Helleborus* L., but also within the same species only from different aspects.

Material and method

The study on the influence of the extract of underground plant organs of *Helleborus odorus* Walds. et Kit. on the changes regarding the value of erythrocytes count, concentrations of haemoglobin and hematocrits was carried out on the Wistar type rats. The animals of both sexes were represented, in the age of 2 months, body mass 200 ± 10 g, and divided into 4 groups with 7 rats in each.

Chopped plant material (rhizome and roots of the species *H. odorus* Walds. et Kit.) was extracted in the apparatus for continual extraction according to Soxhlet solvents of increasing polarity, and afterwards by water. Obtained dry extract was dissolved in physiological solution up to a required concentration. Control group of rats was treated by a physiological solution in the quantity of 0,25ml /100g TM (group I). In the trial groups the rhizome and root extract of *H. odorus* Walds. et Kit. (EK) was applied intramuscularly into hind limb in the concentrations of 0,5, 5 or 20 mg/100g TM (groups II, III and IV). After 24h the rats were anaesthetized by ether, and blood samples for analysis taken by cardial puncture.

Haematologic parametres were determined by a whole heparinized blood by means of an automatic haematological analyser Arcus Diatron®, Gmbh Wien, Austria. The analysis of statistical significance of the differences among mean values of haematological parametres in studied groups was performed by Student t-test.

Results and discussion

Table 1 shows the results obtained in the process of determination the erythrocytes count, concentration of haemoglobin and haematocrit values of both control and trial groups 24h after the application of the *H. odorus* Walds. et Kit. rhizome and root extract in the concentrations of 0,5, 5 or 20 mg/100g TM.

All trial groups had lower erythrocytes count in blood in relation to a control group, at which significantly lower mean erythrocytes count was recorded in the groups treated with 5mg/100g T.M. or 20 mg/100g T.M. extract of hellebore ($P < 0,01$). Mean values of erythrocytes count in blood collected from trial groups I, II and III are different, but those differences are not statistically significant ($P > 0,05$).

A statistically higher mean value of the haemoglobin blood concentration in the rats from control group in relation to the groups to

which 5mg/100g T.M. or 20mg/100g T.M. extract of hellebore was applied ($P < 0,05$) was confirmed. A statistically significant differences in the haemoglobin concentrations between the trial group which received 0,5mg/100g T.M. extract of hellebore and control group was not observed, and neither was it observed among the trial groups (I, II i III).

Table 1. Erythrocyte number ($10^{12}/L$), haemoglobin concentration (g/L), HCT value (%)

Haematological parameters	I (FR 24h)		II (EK 0,5mg 24h)		III (EK 5mg 24h)		IV (EK 20mg 24h)	
	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Erythrocyte number ($10^{12}/L$)	7,91	0,48	7,29	0,60	6,93	0,50	6,81	0,48
Haemoglobin concentration (g/L)	127,71	8,12	127,43	13,51	111,43	17,09	115,00	11,87
HCT value (%)	36,89	2,19	36,81	3,74	33,29	5,15	32,63	4,34

A mean haematocrit value in the group of rats treated by 20mg/100g T.M. rhizome and root extract of hellebore was significantly lower in relation to the control group ($P < 0,05$). Higher values of haematocrits in control group of rats in relation to trial groups treated by 0,5mg/100g T.M. or 5mg/100g T.M. extract of hellebore were not statistically significant ($P > 0,05$). Among trial groups (I, II i III) statistically significant differences of mean haematocrit values were not established.

In the course of the trial, the established mean erythrocytes count in all studied groups was in the range of reference values stated by *Pritchett and Corning* (2004) ($5,4-8,5 \times 10^{12}/L$) and *Moore* (2000) ($7,25 \pm 0,93 \times 10^{12}/L$ in males and $6,85 \pm 0,61 \times 10^{12}/L$ in females). Our results obtained by determination of erythrocytes count in the blood of rats 24h after application of rhizome and root extract of hellebore are in accordance with the results of some other authors. *Petričić et al.* (1971) state that *H. odoratus* Walds. et Kit. shows weak haemolytical activity which is lower in comparison with other plants of genus *Helleborus* L. *Ristoska et al.* (2002) established that the mean erythrocytes count in the blood of gilts decreased 24h after application of the extract of hellebore, but that it remained within the physiological limits. In piglets, 52 days old, *Tosevski et al.* (2004) established a decrease of 22% in the erythrocytes count on the 14. and 21. day after the application of stinking hellebore extract in relation to values obtained before treatment. In

the same trial, erythrocytes count was within the limits of reference values on the 7. and 14.day after application of the hellebore extract to the piglets in the age of 35 days.

During the trial, the haemoglobin blood concentrations in the control and trial groups of rats were lower than physiological values which according to the statement of *Moora* (2000) amount to 143 ± 10 g/L blood. The results obtained on rats are in accordance with the results obtained by *Tosevski et al.* (2004) in the trial on piglets when they were 52 days old. These authors confirmed the decrease in the haemoglobin concentration by 11% on average. In piglets being 35 days old, on the seventh and fourteenth day after the application of the hellebore extract the haemoglobinaemia within the physiological limits was observed. *Ristoska et al.* (2002) also confirmed that the value of the concentration of haemoglobin remained in the range of reference values after the extract of hellebore was applied on gilts. Haematocrit values in the blood of rats in all investigated groups were somewhat lower than reference values (37-49%) stated by *Pritchett and Corning* (2004).

Conclusion

Intramuscular application of the rhizome and root extract of *Helleborus odorus* Walds. et Kit. can induce a weak haemolysis in the blood of treated rats. Simultaneously, transcutaneous implantation of the rhizome of *Helleborus* L. and intramuscular application of the extract of underground organs of *Helleborus* L. to different kinds of animals can lead to the increased activity of rapid, unspecific mechanisms of immuno response.

HEMOLITIČKA AKTIVNOST EKSTRAKTA RIZOMA I KORENA KUKUREKA (*H. odorus* WALDST. ET KIT.) PRIMENJENOG KOD PACOVA SOJA WISTAR

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Rezime

Fitoterapija se u zdravstvenoj zaštiti životinja veoma intenzivno

primenjuje u profilaktičke svrhe i u cilju lečenja blažih oblika bolesti i bolesti hroničnog toka. Iz tradicionalne, narodne medicine poznato je da kukurek podstiče funkcije efektorskih odbrambenih ćelija i povećava aktivnost brzih, nespecifičnih mehanizama imunskog odgovora. Biljke roda *Helleborus* L. sadrže saponozide koji povećavaju permeabilnosti membrane eritrocita što dovodi do hemolize i gubitka hemoglobina.

U ovom radu ispitali smo uticaj ekstrakta rizoma i korena *H. odorus* Waldst. et Kit. na vrednost broja eritrocita, koncentraciju hemoglobina i hematokritsku vrednost. Pacovima oglednih grupa ekstrakt kukureka je aplikovan intramuskularno u koncentraciji 0,5, 5 ili 20mg/100g TM. Pacovima kontrolne grupe na isti način je aplikovan sterilan fiziološki rastvor u količini od 0,25ml/100g TM.

Ključne reči: *H. odorus* Waldst. et Kit., hemolitička aktivnost, pacovi

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