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USE OF *Petroselinum crispum* AND VITAMIN E TO PROTECT AGAINST CARMOISINE CHANGES IN RATS

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Abstract

Carmoisine is a food coloring found in many foods, with several restrictions. The aim of this work was to evaluate the hematological and biochemical parameters of blood in Wistar albino laboratory rats after oral administration of carmoisine every day for 6 weeks by dissolving the additive in water. The effect of temperature on parsley was also studied, drying it at different temperatures, then observing at what temperature the amount of ascorbic acid was maintained in the greatest proportion. At most haematological parameters, higher values were observed in rats in the 100 mg carmoisine group compared to the control and parsley groups. Similarly, the biochemical parameters analyzed showed higher values in rats in the group receiving 100 mg carmoisine compared to the control group, and parsley administered to rats was able to bring mean values closer to those obtained in the control group.

Key words: *biochemical, blood, carmoisine, FT-IR, hematological parameters, petroselinum crispum, vitamin E.*

INTRODUCTION

Carmoisine is a synthetic food coloring, it is also called azorubin, encoded with E 122, has a brown-red color and belongs to the category of azo dyes (Coroian, 2019). HPLC and HPLC-DAD shall be used for the determination of carmoisine in foods, flavouring substances, alcoholic beverages, fruit drinks, jams, confectionery (Minioti et al., 2007). Carmoisine is a synthetic food coloring specifically used in foods to be heat treated after the fermentation process. Similar to other azo dyes, carmoisine can cause allergies, especially for those with aspirin intolerance. Since it is a histamine releaser, it is not recommended for people suffering from asthma, as it can intensify this disease and adverse effects. Children are not recommended products containing carmiozine and other additives from the category of benzoates, as they can cause hyperactivity syndrome and lack of concentration (EFSA, 2009; Coroian, 2013; 2019). Nouioura et al. (2023) made a complex based on various plants with high antioxidant capacity, including *Petroselinum crispum*. Recent studies by Peshkova et al., 2023, characterize the translocation of copper and gold nanoparticles in *Petroselinum crispum*. Evaluates the influence of hydroalcoholic extract of (*Petroselinum crispum*) for anxiety in rats that were treated with lead acetate (Fatemeh et al., 2023). Studies conducted on *Petroselinum crispum* show that it slows down the aging process of the skin, helps people with low immune systems, is very useful in the diet of people suffering from indigestion, constipation and in case of pancreas problems (Jassim, 2013). Parsley has a very high content of vitamin C, which helps strengthen the immune system, is beneficial for physically exhausted people (IFNB, 2000). Due to the content of vitamins and provitamins it is extremely beneficial for a balanced diet (Heinonen et al., 1989; Coroian, 2019). Exhibits high antioxidant capacity (Jassim, 2013). The effect of antioxidant activity of (*Petroselinum crispum)* and vitamin C, used against oxidative stress, has been reported by (Meister, 1992; Podmode et al., 1998, Nielsen

et al., 1999; Zhang et al., 2006). Characterisation of food additives, risk assessment and interaction with food are provided by (Basu & Gopinatha, 2014; Scotter, 2015; Tofană, 2006; Tomaska & Brooke-Taylor, 2013). The purpose of this paper is to evaluate changes in biochemical and hematological parameters in rats and to evaluate weight oscillations after carmoisine administration and protective use of parsley and vitamin E.

MATERIALS AND METHODS

The experiment was conducted over a period of 6 weeks during which the substances were administered to rats daily. The carmoisine used in the study is presented as a reddish-brown powder with no other substances that may influence the test results. It was administered orally to the animals in the experiment. Carmoisine being water soluble, it was dissolved in water and then administered to rats. The maximum allowable dose for the human body is 4 mg/kg body weight. Since carmoisine toxicity is weakly expressed in amounts slightly higher than the maximum allowable dose, over a relatively short period of time, we administered 60 and 100 mg carmosine per day in this study. Parsley was used in this experiment due to the high amount of ascorbic acid it holds. This, together with vitamin E, has antioxidant action, thereby reducing the effects of oxidative stress caused by carmoisine. The parsley was dried at room temperature at 40ºC, 70ºC and 90ºC respectively to avoid oxidation of vitamin C in parsley. After the parsley was dried, it was shredded, then the rats were fed. Vitamin E was stored in the form of gelatin capsules at 21ºC in a dark space. From inside these capsules, vitamin E was extracted using disposable syringes and, after a short time, administered to rats. There were 4 groups in the study: 1 – control group; 2- the group to which I administered 60 mg carmoisine; 3 – the group to which I administered 100 mg carmoisine + parsley and vitamin E; 4 – the group to which I administered 100 mg carmoisine.

Animals. Rats in the experimental groups were weighed at the beginning and end of the experiment to observe any changes in weight for each individual. A total of 5 rats were used for each batch in the experiment. Before the experiment began, the rats were acclimatized in the laboratory animal unit at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. The research project was approved by the Institutional Committee for Research Ethics, nr. 110, and was authorized by the sanitary veterinary authority, Cluj-Napoca, under no. 8187.

Hematological and biochemical analysis of blood. For the analysis of hematological parameters, the automatic hematology analyzer Abacus Junior was used, which uses 25 µl of blood for analysis. To determine the biochemical parameters, immediately after collection the blood was subjected to centrifugation in order to separate the serum. For biochemical analysis, the semi-automatic screen point analyzer with reagents was used (model: STAT-FAX 1904 Plus, GMI, Inc. 6511, Minnesota, 55303 USA).

FT-IR Analysis. The FT-IR/FT-Raman 4100 Jasco spectrometer (the resolution of the obtained spectra was set to 4 cm^{-1}), was used for parsley analysis. The beam divider in KBr was used for analysis and the method of pastylating the sample in powder form (5 mg) with potassium bromide (300 mg) by pressing at 10 t/cm^2 and the MIR probe for non-destructive testing on the spectral range 350-4500 cm^{-1} was used. With OPUS software, version 6.0 spectra were processed. The parsley samples were subjected to a heat treatment at different temperatures, namely: 40^0C, 70^0C, 90^0C, but also dried parsley at room temperature, to observe changes depending on temperature.

RESULTS AND DISCUSSIONS

By weighing at the beginning and end of the experiment, differences in weight can be observed by comparing the control group with the other groups receiving carmoisine. Batch 2, which received carmoisine, parsley and vitamin E, did not undergo major weight loss. In contrast, the groups receiving 60 mg carmoisine and 100 mg carmoisine, respectively, experienced significant weight loss. Also, at the end of the experiment, they showed quieter behavior and lacked energy. From the data obtained by weighing at the beginning and end of the

experiment, differences in weight are observed by comparing the control group with the other groups receiving carmoisine. The group that received carmoisine, parsley and vitamin E did not undergo major weight loss. In contrast, groups receiving 60 mg carmoisine and 100 mg carmoisine experienced significant weight loss. Also, at the end of the experiment, they showed quieter behavior and lacked energy.

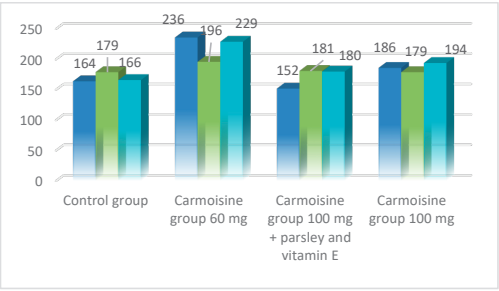

Figure 1. The weight of rats at the beginning of the experiment (g)

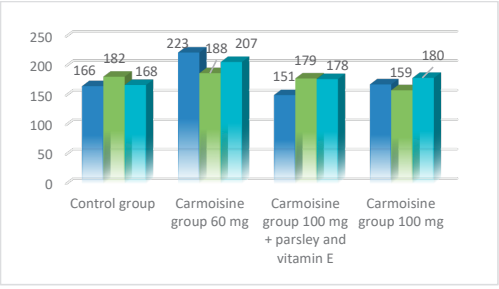

Figure 2. Weight of rats at end of experiment (g)

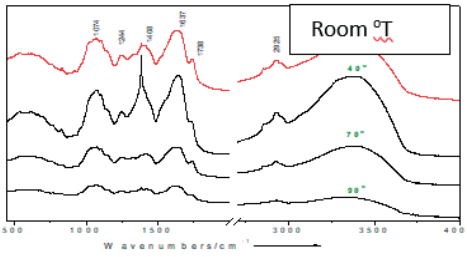

Figure 3. FT-IR spectra for parsley under the influence of temperature

Analyzing the FT-IR spectrum obtained from dried parsley, shown in figure 3, we can see a high pick intensity from 1637 cm^{-1} of the spectrum obtained from dried parsley at room temp and dry parsley at 40°C. This intensity may be due to the bond C=C. It can be seen that this band decreases in intensity with increasing temperature. We can also observe a decrease in intensity of the strip from 1075 cm^{-1} to the C=O bond. Also, the bands from 1244 and 1403 that are quite obvious in the case of dried parsley lose their intensity depending on the temperature to which the plant has been subjected. It can be noted that the band from 2925 cm^{-1} attributed vibrationally to the CH group is almost non-existent in the spectrum obtained from dried parsley at a temperature of 90^0C.

Evaluation of haematological parameters in rats after carmoisine administration

Tables 1 to 4 show the mean values for haematological indices in the blood analysed from the rats in the experiment.

Table 1. Mean values and variability of haematological parameters in control rats

Parameter	Unit	Control group	
		$X \pm S_x$	V%
WBC	10^9/l	5.042±2.15	19.35
LYM	10^9/l	3.916±1.62	22.58
MID	10^9/l	0.64±0.08	29.25
GRA	10^9/l	1.6±0.66	21.04
LY	%	60.40±4.21	4.47
MI	%	5.20±0.10	4.51
GR	%	21.80±0.92	9.47
RBC	10^12/l	7.636±0.17	5.04
HGB	g/l	133.40±0.75	1.25
HCT	%	38.01±0.29	1.70
MCV	fl	42.60±1.60	8.40
MCH	pg	14.380±0.25	3.95
MCHC	g/l	332±11.62	7.83
RDWc	%	21.32±1.06	11.09
PLT	10^9/l	716.60±8.45	2.64
PCT	%	0.59±0	1.2
MPV	fl	6.5±0.07	2.43
PDWc	%	32.62±0.26	1.81

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 2. Mean values and variability of haematological parameters in rats in the carmoisine 60 mg group

Parameter	Unit	Carmoisine group 60 mg	
		$X \pm S_x$	V%
WBC	10^9/l	5.99±1.03	38.60
LYM	10^9/l	4.08±0.54	29.32
MID	10^9/l	0.70±0.18	57.38
GRA	10^9/l	1.90±0.42	49.55
LY	%	62.60±1.54	5.49
MI	%	6.26±0.15	5.49
GR	%	28.26±2.02	15.98
RBC	10^12/l	7.99±0.28	7.94
HGB	g/l	130.60±2.66	4.55
HCT	%	39.79±0.56	3.17
MCV	fl	43.40±1.17	6.01
MCH	pg	14.6±0.73	11.11
MCHC	g/l	330.80±2.22	1.50
RDWc	%	21.74±0.21	2.17
PLT	10^9/l	759.80±13.27	3.90
PCT	%	0.66±0.02	5.59
MPV	fl	6.60±0.12	4.15
PDWc	%	32.62±0.14	0.98

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 3. Mean values and variability of hematological parameters in rats of the carmoisine group 100 mg + parsley and vitamin E

Parameter	Unit	Carmoisine group 100 mg + parsley and vitamin E	
		$X \pm S_x$	V%
WBC	10^9/l	6.49±0.61	20.96
LYM	10^9/l	3.99±0.52	29.18
MID	10^9/l	0.65±0.16	57.02
GRA	10^9/l	1.37±0.28	45.49
LY	%	64.87±2.11	7.26
MI	%	6.72±0.15	4.87
GR	%	29.84±0.87	6.51
RBC	10^12/l	8.89±0.19	4.91
HGB	g/l	139.60±1.60	2.56
HCT	%	42.87±0.50	2.62
MCV	fl	47.8±1.24	5.81
MCH	pg	15.66±0.28	3.98
MCHC	g/l	334.80±6.61	4.42
RDWc	%	22.8±0.21	2.06
PLT	10^9/l	768.40±10.75	3.13
PCT	%	0.66±0.02	6.95
MPV	fl	6.66±0.12	3.92
PDWc	%	33.46±0.32	2.11

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 4. Mean values and variability of haematological parameters in rats of the carmoisine group, 100 mg

Parameter	Unit	Carmoisine group 100 mg	
		$X \pm S_x$	V%
WBC	10^9/l	12.02±0.54	10.11
LYM	10^9/l	4.59±0.26	12.58
MID	10^9/l	1.07±0.21	44.16
GRA	10^9/l	1.96±0.11	12.93
LY	%	70.26±4.70	14.96
MI	%	7.10±0.10	3.30
GR	%	33.42±0.57	3.83
RBC	10^12/l	9.22±0.27	6.44
HGB	g/l	139.4±1.08	1.73
HCT	%	43.17±0.14	0.72
MCV	fl	53.60±2.01	8.41
MCH	pg	17.26±0.45	5.79
MCHC	g/l	325.2±3.72	2.56
RDWc	%	23.72±0.84	7.92
PLT	10^9/l	781.40±7.70	2.20
PCT	%	0.698±0.09	29.02
MPV	fl	7±0.07	2.26
PDWc	%	33.740±0.24	1.62

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

The WBC showed highest values in group 3, which received 100 mg carmoisine, with a value of 12.02±0.54. The WBC showed highest values in group 3, which received 100 mg carmoisine, with a value of 12.02±0.54. Parsley along with vitamin E were able to keep the value of white blood cells at an optimal value. LYM shows similar values in the control group compared to the group which, in addition to carmoisine, was given parsley together with vitamin E, but the group given only 100 mg carmoisine showed higher values 4.59±0.26. The same applies to the MID parameter, where the control group and group 2 show low differences (0.64±0.08, 0.65±0.16) and group 3 show high values (1.07±0.21) exceeding the normal value of this parameter of 0.98. Although there is a difference between granulocyte values in the four groups of rats, they do not exceed normal values, as well as for LY, where group 3 (70.26±4.70) shows the highest values not exceeding the limit of 97.

As for hematocrit, the values between the four groups are close, none of them exceeds the normal limit of this parameter. For MI mean value for control group was 5.20±0.10, for group 60 mg carmoisine mean value was 6.26±0.15, for group 2 with carmoisine and vitamins was 6.72±0.15 and for group 100 milligram carmoisine was 7.10±0.10. Haemoglobin (HGB) and mean red blood cell haemoglobin (MCHC) concentration were not affected by the food additive administered to the rats in the experiment, the values of these parameters not changing with the amount of carmoisine ingested by the rats.

Haematological parameters showed higher values in rats in the carmoisine group compared to the control group and the one given parsley and vitamin E, it can be concluded that the food additive can alter haematological parameters even for a short period of time.

Evaluation of biochemical parameters in rats after carmoisine administration.

Biochemical profile in the blood is carried out to assess the health of an organism. By analyzing biochemical constituents, various pathophysiological states and metabolic disorders in animals can be diagnosed (Green et al., 1992). Tables 5 to 8 show mean values of biochemical parameters of blood collected from rats in the experiment (control group, group 1 with carmoisine 60 mg, group 2 with 100 mg carmoisine+parsley and vitamin E, group 3 with 100 mg carmoisine). Following analysis of biochemical parameters, it can be seen that mean AST (U/l) increased in group 3, which ingested 100 mg carmoisine (131.6±0.75), compared to the control group, where the mean value was (115.6±2.56). Regarding the ALT parameter (U/l) it can be seen that there are no large differences between the batches, but, as in the case of AST, the highest values are presented by the group with 100 mg carmoisine (64±1.30). In the case of glucose, it can be seen

that there are no differences between the control group (224.6±2.50) and group 2 (224.6±2.50), despite the fact that the second group received 100 mg carmoisine and parsley and the control group did not receive this additive.

The carmoisine 60 mg group had a decrease in this parameter (206.4±3.08) compared to the control group, and group 3 had an increase in this value (242±2.51), thus outperforming all other groups.

Related to cholesterol (mg/dl), triglycerides (mg/dl) and creatinine (mg/dl) it can be said that the differences between the groups are insignificant.

Cholesterol was little influenced by administration of the additive to rats. In contrast, triglycerides increased slightly comparing the control group (57.8±1.39) with group 3 (59.4±1.36). However, administration of parsley and vitamin E seems to decrease the value in group 3, to 59±1.3.

Table 5. Average values and variability biochemical parameters in control rats

Parameter	Unit	Control group	
		$X \pm s_x$	V%
AST	U/l	115.6±2.56	4.95
ALT	U/l	59.80±1.39	5.21
Glucose	mg/dl	224.6±2.50	2.49
Cholesterol	mg/dl	68.6±1.03	3.36
Triglycerides	mg/dl	57.8±1.39	5.39
Creatinine	mg/dl	0.48±0.01	5.27

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Creatinine also underwent slight oscillations, but the differences between the batches are insignificant. In a study conducted by Amin & Hameid (2010) was tested the influence of carmoisine on parameters such as ALT, AST, ALP, creatinine, glucose. After administration of food coloring over a period of 30 days, they noticed a significant increase in the mean value for these parameters (Amin & Hameid, 2010). It should be noted that by administering carmoisine to experimental rats, the value of the GR parameter analyzed increased significantly from the value of 21.80±0.92 of the control group to the value of 33.42±0.57 of the rats of group 3. Groups 1 and 2 also showed close values despite the different amounts of carmoisine administered to each group.

Table 6. Mean values and variability of biochemical parameters in rats in the carmoisine 60 mg group

Parameter	**Unit**	**Carmoisine group 60 mg**	
		$X \pm s_x$	**V%**
AST	U/l	119.2±4.07	7.63
ALT	U/l	60±1.48	5.53
Glucose	mg/dl	206.4±3.08	3.33
Cholesterol	mg/dl	67±0.71	2.36
Triglycerides	mg/dl	51±0.71	3.10
Creatinine	mg/dl	0.47±0.01	2.45

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Table 7. Mean values and variability of biochemical parameters in rats of the carmoisine group (100 mg + parsley and vitamin E)

Parameter	Unit	Carmoisine group 100 mg + parsley and vitamin E	
		$X \pm s_x$	V%
AST	U/l	128.2±0.66	1.16
ALT	U/l	62±0.84	3.02
Glucose	mg/dl	224.6±2.50	2.49
Cholesterol	mg/dl	69.8±0.86	2.76
Triglycerides	mg/dl	59±1.3	4.94
Creatinine	mg/dl	0.48±0.01	5.19

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Table 8. Mean values and variability of biochemical parameters in rats of the carmoisine group

Parameter	Unit	Carmoisine group 100 mg	
		$X \pm s_x$	V%
AST	U/l	131.6±0.75	1.27
ALT	U/l	64±1.30	4.56
Glucose	mg/dl	242±2.51	2.32
Cholesterol	mg/dl	70.2±0.37	1.19
Triglycerides	mg/dl	59.4±1.36	5.13
Creatinine	mg/dl	0.5±0.01	2.98

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Amin & Hameid (2010) observe an increase for AST, ALT, alkaline phosphatase, urea, creatinine and albumin, for the group treated with carmoisine in low quantity and at the same time these values increasing significantly in those treated with high dose carmoisine. Gaunt et al. (1967) note that carmoisine negatively affects and alters biochemical markers in vital organs such as liver and kidneys at both lower and high doses. Studies on the effect of carmoisine on the biochemical profile, evaluation of oxidative stress in laboratory animals and in the womb, as well as acute and chronic toxicity studies, have been conducted

by (Mason et al., 1974; Holmes et al., 1974; Ford et al., 1987; Amin & Hameid, 2010; Lamia et al., 2016; Coroian, 2019).

CONCLUSIONS

Most of the parameters analysed showed higher values in rats in the carmoisine group compared to the control and groups that also received parsley and vitamin E. The way and temperature of drying can influence the chemical structure of parsley and implicitly its properties.

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