Studii şi Cercetări	Biologie	11	141 - 145	Universitatea din Bacău	Mai 2006

HEPATOPROTECTIVE EFFECTS OF SOME ANTOCYANIC VEGETAL EXTRACTS

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***Key words**:antocyanic extracts, membrane potential, rH, hepatoprotective effects*

INTRODUCTION

As known, the various maladies of the liver, alongwith the action of some toxic agents manifested at the level of this organ, determine a series of characteristic modifications in the morphophysiological parameters of the hepatic cells [5, 6, 8, 11].

A series of previous studies have been devoted to the characteristic modifications observed in alcoholic hepatopathy, expressed by immunulogical perturbations, perturbance of some enzymes' activity [2, 6, 8, 10], modification of hepatocytes' membrane potential and of the redox potential [6].

Another observation referred to the fact that the action of the allylic alcohol induces a series of specific modifications at the level of liver's cells, similar to those produced in certain hepatic affections, and expressed through irreversible membranary depolarizations, modifications of the redox potential and perturbations of the ionic ratios etc. [7, 8]. Nevertheless, treatments with some acetylated polyollic products reduced such types of peturbations to a considerable extent [8].

Other investigations showed that certain polyphenolic vegetal extracts have a positive action at cellular level [4, 5, 9, 11], re-establishing the normal electrical charge of the muscular and hepatic cells membrane modified under the action of certain toxic agents [7, 8], as well as important cytostatic and antitumoral properties [10].

The present study presuming the previous investigations, follows the action of a vegetal extract of antocyanic nature on the liver of some laboratory animals intoxicated with allylic alcohol, for putting into evidence some possible hepatoprotecting effects, on considering antocyans' polyphenolic chemical structure [1, 3].

MATERIAL AND METHOD

The experiments performed *in vitro* liver of frog (*Rana ridibunda*, Pall), have utilized two groups of preparations: a reference normal group, and another one, intoxicated with 3% allylic alcohol, in a normal Ringer (NR) physiological solution.

Both batches have been treated with a total aqueous extract, of antocyanic nature, made of pink *Hibiscus sp.* flowers [1].

Different concentrations of extract [HIB] in normal Ringer solution have been applied, as follows: 0.001%, 0.005%, 0.01%, 0.1%, 0.5% and 1%. As reference agent, regarding the similar cellular effects, 0.0014% silimarine [SIL] – a polyphenolic pharmaceutical product of vegetal origin, recommended in the treatment of some hepatic affections [1, 12] – has been utilized.

Evidencing of the direct effects of the agents on the hepatic cells involved followed of the dynamics of the membrane potential, known as expressing the physiological condition of the hepatocytes [6, 8], the determinations being made by means of glass intracell microelectrodes.

Starting from the antioxidating properties of the antocyanic products [3, 5, 11], there have been also determined, potentiometrically, the values of the redox potential and of the extracts' pH. Quantification of the redox effects involved calculation of the rH values on the basis of the E_h and pH values, according to the relation of Clark:

$$rH = \frac{E_h + 0.058 + 250}{0.029} \text{ (volts) [13]}$$

The references batch of hepatic preparations has been treated initially with an antocyanic extract of various concentrations, for putting into evidence the effects upon normal cells and then with NR, for establishing the degree of recovery of the membrane potential values.

For analyzing the possible hepatoprotecting effects of the antocyanic extracts, another batch of hepatic preparations had been initially intoxicated with 3% allylic alcohol in NR, after which it was treated with the extract. In both batches, each experimental phase had a duration of 60 minutes.

For the establishment of the antocyanic extract's compounds, this was subjected to HPLC analysis (Fig. 1).

The initial membrane potential values have been calculated statistically, with Student test. Some comparative values have been calculated as percents *versus* the normal initial values or *versus* the effect of the corresponding treatment agent.

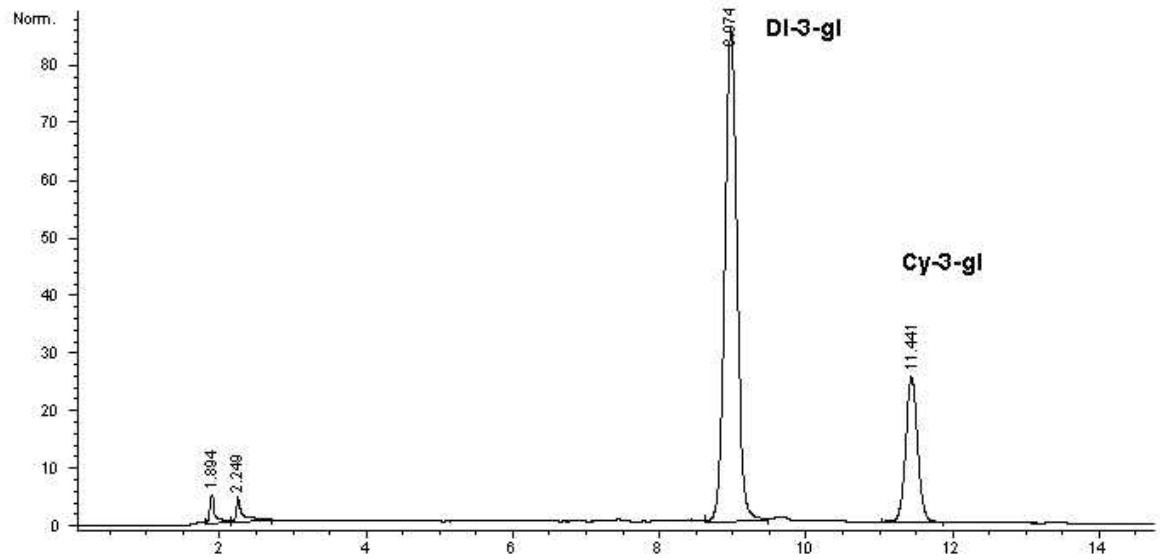

Fig. 1. Extract's HPLC - antocianic components: Dl-3-gl – delfinidin-3-monoglicoside; Cy-3-gl – cianidin-3- monoglicoside

RESULTS AND DISCUSSIONS

HPLC analysis of the *Hibiscus* aqueous total extract evidences two major monoglicoside components of antocianic extract, containing delfinidin and, respectively, cianidin (Fig. 1), which act together upon hepatic cell.

The normal resting potential (NRP) of the hepatic cells recorded initial values ranging between 28.55 mV and 33.20 mV (Tables 1 and 2).

Table 1. Bioelectrical effects, after 60 min action, of different concentrations of *Hibiscus* extract (HIB) and of silimarine (SIL) upon normal hepatic cells (NRP = normal resting potential; NR = normal Ringer solution; mV = milivolts)

Nr.	Agent' concentration (%)	NRP $\bar{X}$±SE (mV)	Agent' effect (±% from NRP)	NR effect after agent	
				(±% from NRP)	(±% from agent effect)
1	SIL 0.0014	32.16±0.23	+11.75	+3.61	+69.31
2	HIB 0.001	30.17±0.32	+6.83	+1.03	+84.95
3	HIB 0.005	32.18±0.23	+8.45	+2.24	+73.53
4	HIB 0.01	31.51±0.37	+11.29	+2.28	+79.77
5	HIB 0.1	31.73±0.25	+9.26	+3.62	+60.88
6	HIB 0.5	33.20±0.31	–5.60	+2.17	+138.71
7	HIB 1	30.37±0.17	–7.31	+0.46	+106.31

Table 2. Bioelectrical effect (RP recovery – %) of *Hibiscus* extract (HIB) în different concentrations and of silimarine (SIL) upon depolarized by 3% allylic alcohol (ALL) hepatic cells,after 60 min action

Nr.	Agent' concentration (%)	NRP $\bar{X}$±SE (mV)	ALL' effect (depol = % from NRP)	RP recovery in NR after ALL	
				(±% from NRP)	(±% from ALL depol)
1	ALL 3%	28.55±0.38	–7.88	–4.55	+43.55
2	SIL 0.0014	30.84±0.43	–11.25	+0.29	+102.59
3	HIB 0.001	31.17±0.34	–11.42	–5.96	+47.70
4	HIB 0.005	32.05±0.28	–12.11	–7.23	+46.90
5	HIB 0.01	32.08±0.31	–10.63	–2.64	+75.07
6	HIB 0.1	31.62±0.30	–9.58	–0.60	+93.73
7	HIB 0.5	30.73±0.26	–7.84	–7.09	+9.54
8	HIB 1	31.12±0.32	–8.67	–9.19	–5.92

The reference batch of normal hepatic preparations was treated with the *Hibiscus* extract (HIB), in pre-established concentrations.

The bioelectrical effects of the HIB extract have been compared to these attained on another series of hepatic preparations treated with a reference hepatoprotecting drug – silimarine, in clinical concentrations of 0.0014% [12].

The results obtained evidenced a dual bioelectrical effect, which depends on the agents' concentrations. Consequently, silimarine and the low concentrations of antocyanic extract (0.001%–0.1%) cause an increase of the membranary electrical charge (hyperpolarization), while the high concentrations of extract (0.5%–1%) provoke decreases in the values of the membrane potential (depolarization), the amplitude of both effects depending on the agent's concentration (Table 1).

The most intense hyperpolarization effect is produced by silimarine (11.75% from NRP). The hyperpolarizing effect of the antocyanic extract increases slightly with concentration, from 6.83% in

NRP, at a concentration of 0.001%, up to 11.29% from NRP, at a 0.01% concentration value (which is an effect similar to that of silimarine). At higher concentrations, hyperpolarization decreases (9.26% from NRP at a concentration of 0.1%), being subsequently transformed into a membrane depolarization which increases with the concentration of the extract, as follows: 5.60% from NRP at concentration of 0.5% and, respectively, 7.31% at 1% concentration (Table 1).

All these results evidence several significant aspects. Thus, one may first observe that the specific bioelectrical effect of the antocyanic extract is manifested by membranary hyperpolarization, characteristic to low concentrations. At the same time, even the membranary depolarization induced by higher concentrations is rapidly reversible, as, after a 60 minutes washing of the preparations with normal Ringer without extract, hyperpolarization is to be observed, alongwith lowering in the concentration of agent bound in the membranary structure (Table 1). One may also observe that the hyperpolarizing effect is extremely persistent, as, on washing of the hepatic preparations with Ringer without agent, for 60 minutes, the reduction in hyperpolarization is extremely slow, in the case of HIB, the value recorded ranging between 84.95%, at 0.001% concentration, and 60.88% at 0.1% concentration, while, in the case of silimarine, it represents 69.31% of the maximum hyperpolarization (Table 1).

Such an effect evidences a strong bonding of the polyphenolic agents to the structure of the cellular membranes, whisc is to be correlated with their properties of membrane stabilizers, with the reduction of permeability and with the influence upon the Na^+–K^+-ATP-ase involved in their pharmacological effects [2,7,12].

Important aspects also result from the data expressing the action of the antocyanic extracts upon the hepatic preparations intoxicated with allylic alcohol.

The action of allylic alcohol on the hepatic cells results in a membranary depolarization with an amplitude of 7.84%–12.11% of the NRP initial value (Table 2). The action of the allylic alcohol affects quite serriously the structure and properties of the hepatic cells' membranes, so that, when the hepatic preparations are washed with NR without allylic alcohol, the recovery of NRP is extremely slow, after 60 minutes depolarization being reduced with only 43.55% of its maximum value (Table 2).

Treatment of the hepatocytes depolarized by allylic alcohol with antocyanic extracts induces an obvious action of the latter ones towards the recovery of the normal properties of the hepatic cells, as expressed by the recovery of NRP, in ratios depending on the extracts' concentration. Consequently, the strongest positive effects in this respect are those of the 0.01% concentration, seen as reducing depolarization with 75.07%, followed by the 0.1% one, which reduces depolarization with 93.73% (Table 2). 1% HIB amplified, still, the allylic depolarization with 5.92% (Table 2).

Nevertheles, silimarine reduces allylic alcohol depolarization on the whole up to inducing a 2.59% hyperpolarization of the membranes treated with allylic alcohol. Although much lower than those of silimarine, the effects of the antocyanic extracts are nevertheless comparable to those of the mentioned hepatoprotecting drug.

Consequently, such data may act as proofs supporting the fact that the antocyanic extracts obtained from *Hibiscus* contain biologically-active principles, being thus capable of re-establishing the normal structural-funcțional condition of the hepatic cells, perturbed under the action of some noxious agents, such as the allylic alcohol [4, 5, 11].

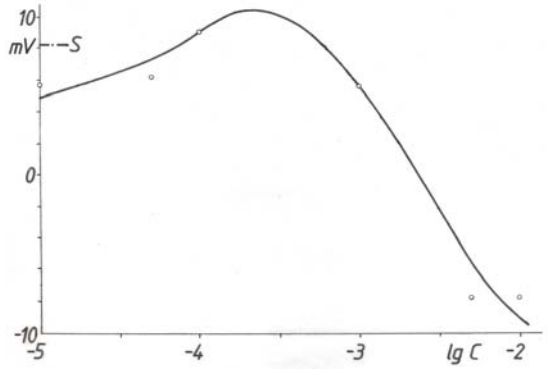

Fig. 2. Repolarization - concentration correlation of the extract effects in normal liver

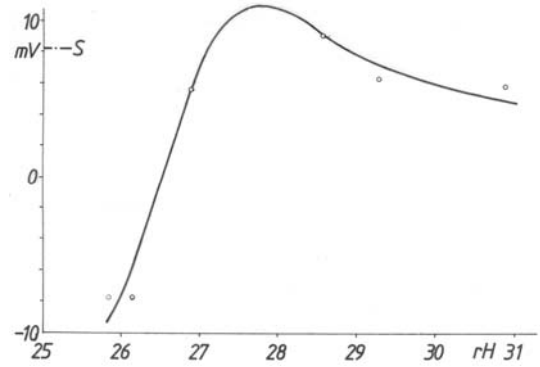

Fig. 3. Repolarization - rH correlation of the extract effects in normal liver

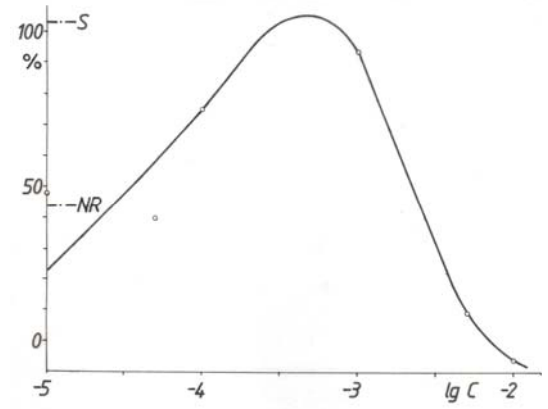

Fig. 4. Repolarization – concentration correlation of the extract effects in allyl alcohol depolarized liver

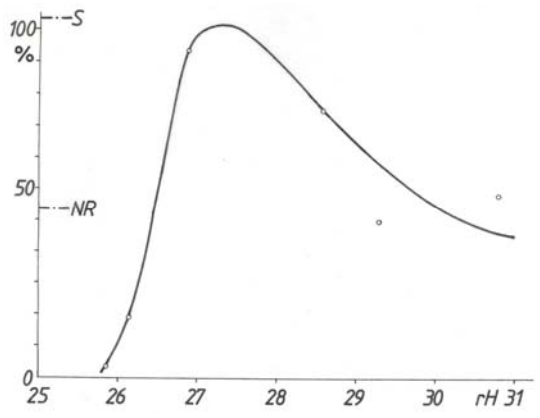

Fig. 5. Repolarization - rH correlation of the extract effects in allyl alcohol depolarized liver

This assertion is tracked up, too, by the existing data on the redox properties of the antocyanic extracts [3, 4, 11]. If considering that a value of 28.3 V represents the neutral rH, that values between 0–28.3 represent the reducing domain and that values between 28.3–42.4 V represent the oxidating domain, the general observation made was that the antocyanic extracts have a slightly reducing rH, which is actually specific to the animal organisms [13]. Nevertheless, the rH values depend on the extract's concentration, that is they decreases gradually with the increase of the latter, symmetrical graphical representations being thus obtained (Figs. 2 to 5).

At the same time, one may also observe that the allylic alcohol induces an ample rH modification (32.6048 V), which may be nevertheless buffered by the antocyanic extracts. Consequently, by plotting graphically the repolarization–concentration correlation and, respectively, the repolarization–rH one, when treatments with antocyanic extracts are applied to the normal hepatic preparations (Figs. 2, 3) and, equally to the preparations intoxicated with allylic alcohol (Figs 4, 5), a common domain of optimum values – expressing the antocyans' capacity of acting as redox modulators – is obtained. Another observation is that such rH values of the antocyanic extracts are similar to those of the hepatoprotecting drug silimarine.

On the basis of all these considerations, a quite interesting conclusion may be drawn as to the properties of the red wine – starting from its considerably high content of antocyans [3, 4, 9, 11]. Therefore, one may appreciate that – as a result of their hepatoprotecting, antioxidative and redox modulating properties – the antocyans contained in wine exercise a protecting effect on the hepatic cells, against the noxious cellular actions of the ethylic alcohol also present in wine, which contributes to the explanation of the so-called "French paradox" [9], on a series of positive effects induced by a moderate consumption of wine.

CONCLUSIONS

The *Hibiscus* antocyanic extract manifests specific bioelectrical effects on the normal hepatic cells, inducing their hyperpolarization at low concentrations (0.001%–0.1%), as well as their depolarization – at high concentrations (0.5%–1%). At the same time, the extract favourizes normalization of the membrane potential of the hepatic cells intoxicated with allylic alcohol, and influences, in positive manner, cells' redox potential.

The data obtained on the effects of *Hibiscus* extract on both normal and allylic alcohol-intoxicated hepatic cells are indicative of the hepatoprotective and redox modulating properties of the active principles of antocyanic nature contained in such extracts, which recommends their possible pharmacological utilization.

REZUMAT

S-au studiat efectele unor extracte apoase antocianice de *Hibiscus sp.* în concentrații diferite asupra celulelor hepatice de broască (*Rana ridibunda*, Pall.) normale şi intoxicate cu alcool alilic 3%, determinându-se potențialul electric membranar şi potențialul redox (rH), ca markeri fiziologici celulari. La ficatul normal, concentrațiile mici de extract determină o hiperpolarizare membranară a hepatocitelor, iar cele mari induc o depolarizare. Celulele intoxicate cu alcool alilic se depolarizează ireversibil. Concentrațiile mici de extract (0,001%–0,1%), dar nu şi cele mari (0,5%–1%), refac potențialul membranar şi starea fiziologică a celulelor intoxicate, ceea ce indică o serie de proprietăți hepatoprotectoare ale extractului antocianic, cu posibile utilizări farmacologice. Efectele bioelectrice ale acestor extracte se corelează cu caracterul lor redox, ceea ce exprimă proprietățile lor de modulator redox.

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