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## SHORT COMMUNICATION

# Protective activity of an anthocyanin-rich extract from bilberries and blackcurrants on acute acetaminophen-induced hepatotoxicity in rats

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### ABSTRACT

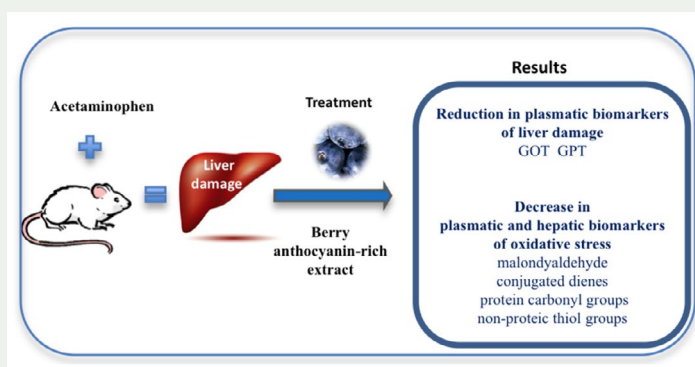
Acetaminophen (N-acetyl-p-aminophenol, APAP) overdose can produce fatal centrilobular hepatic necrosis in humans. The present study attempted to investigate the protective effect of an anthocyanin-rich extract from bilberries and blackcurrants (AE) against APAP-induced acute hepatic damage in rats. Treatment with AE normalised blood activities of glutamate oxaloacetate and glutamate pyruvate transaminase and prevented APAP-induced plasmatic and tissutal alterations in biomarkers of oxidative stress, probably due to various bioproperties of the components of the extract.

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Acetaminophen;  
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anthocyanin; oxidative stress;  
biomarkers



## 1. Introduction

Acetaminophen (N-acetyl-p-aminophenol, APAP) is a well-known antipyretic and analgesic agent with only weak anti-inflammatory properties, remarkably safe in terms of unwanted side effects at therapeutic doses, while its overdose can produce fatal centrilobular hepatic necrosis in humans (Bessemers & Vermeulen 2001). The metabolic APAP biotransformation involves limited conjugation ability, in particular, its glucuronidation and sulphation. At high

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doses, a part of APAP is oxidised by the cytochrome P450, leading to the formation of an electrophilic intermediate, N-acetyl-p-benzoquinone imine (NAPQI). At non-toxic doses, the metabolite is efficiently detoxified by reduced glutathione (GSH); toxicity occurs when the quantity and rate of NAPQI formation overcomes glutathione supply and regeneration (McGill & Jaeschke 2013). The availability of APAP in pharmacies without the need of a prescription has led to it being kept in many homes and it is, therefore, not surprising that it is regularly involved in episodes of accidental and deliberate self-poisoning causing life-threatening liver damage and associated diseases. Thus, it is evident that finding sustainable therapeutic approach to minimising drug-induced liver damage has attracted attention in the recent years.

Many plant antioxidants, intaken through the daily diet or plant-derived dietary supplements, have been shown able to prevent free radical-related diseases by counteracting cell oxidative stress due to their antioxidant capability but, more importantly, by affecting cell signalling pathways (Speciale et al. 2011). Several polyphenols and flavonoids are known to be endowed with protective effects against tissue damage caused to the liver by APAP intoxication; however, as regards this issue, there are really few data about anthocyanins, coloured flavonoids responsible for the blue, purple and red colour of many plant tissues, and present in considerable amount in the human diet (Ali et al. 2003; Horbowicz 2008; Wang et al. 2014). Therefore, in this study we aimed to evaluate the possible beneficial effects of a dry extract enriched in anthocyanins (AE) from blackcurrants and bilberries against acute hepatotoxicity induced by high doses of APAP in rat. In particular, we wanted to clarify whether the protective effect of the AE could be achieved by administering the extract in two doses, one before and one after APAP administration, or if the sole treatment after APAP might be sufficient.

## 2. Results and discussion

The treatment with APAP (3 g/kg orally) produced a significant increase in plasma glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (GOT and GPT) levels in comparison with the control group (Table 1A), so confirming that oral administration of a high dose of APAP in rats produces an evident hepatotoxic effect. This effect could be attributed to hepatocyte necrosis that causes loss of cell transaminases. The significant decrease of transaminases in the groups treated with the AE demonstrates its good hepatoprotector effect. In fact, AE administration produced a statistically significant decrease in plasma levels of GOT and GPT which returned similar to those of controls (Table 1A), demonstrating that also the sole treatment with the AE after APAP administration might be sufficient to protect against APAP-caused hepatocyte loss of transaminases. Finally, AE administration in normal rats (not treated with APAP) did not modify plasma transaminase levels, as well as all measured markers of oxidative stress (data not shown). To test the condition of oxidative stress induced by APAP and the protective effect of AE, we valued the changes, in the blood or in the liver, of several biomarkers of lipid peroxidation and protein oxidation (malondyaldehyde, MDA; proteins' carbonyl groups, CG; conjugated dienes, CD), as well as the hepatic concentration of total, proteic and non-proteic SH groups (T-SH, P-SH and NP-SH).

A significant increase in plasma and liver MDA levels and in liver CD content was measured in rats given APAP administration (Table 1A and B). AE administration has shown a protective effect against APAP peroxidative damage, but in this case, AE appeared less efficient in

**Table 1.** (A). Circulating levels of malondialdehyde (MDA), protein carbonyl groups (CG), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT). (B). Hepatic levels of malondialdehyde (MDA), protein carbonyl groups (CG), conjugated dienes (CD), total thiol groups (T-SH), non-protein thiol groups (NP-SH) and protein thiol groups (P-SH). Data represent mean $\pm$ SD of 5 animals per group; each determination was performed in triplicate.

A						
Group	MDA nmol/ml	CG nmol/mg protein	GOT IU/L	GPT IU/L		
I	0.910 ± 0.16	0.368 ± 0.02	90.79 ± 15.50	38.64 ± 10.04		
II	2.061 ± 0.24*	0.489 ± 0.07*	182.66 ± 35.56*	98.53 ± 17.17*		
III	1.250 ± 0.27 <sup>‡</sup>	0.338 ± 0.05 <sup>§</sup>	101.37 ± 4.88 <sup>§</sup>	42.12 ± 4.32 <sup>§</sup>		
IV	1.262 ± 0.28 <sup>¶</sup>	0.487 ± 0.12	103.15 ± 38.76 <sup>¶</sup>	39.49 ± 3.03 <sup>§</sup>		
B						
Group	MDA nmol/mg protein	CG nmol/mg protein	CD nmol/mg protein	T-SH nmol/mg protein	NP-SH nmol/mg protein	P-SH nmol/mg protein
I	0.45 ± 0.08	0.27 ± 0.10	1.20 ± 0.24	39.54 ± 9.01	1.37 ± 0.32	38.17 ± 6.26
II	0.68 ± 0.06*	0.51 ± 0.06*	2.45 ± 0.39*	23.34 ± 4.54*	0.67 ± 0.19*	22.67 ± 4.57*
III	0.48 ± 0.03 <sup>§</sup>	0.29 ± 0.09 <sup>§</sup>	1.49 ± 0.15 <sup>§</sup>	26.00 ± 1.92*	1.42 ± 0.31 <sup>§</sup>	24.58 ± 2.35*
IV	0.58 ± 0.12	0.38 ± 0.08 <sup>¶</sup>	1.88 ± 0.22 <sup>¶</sup>	24.97 ± 1.82*	0.62 ± 0.23*	24.35 ± 2.40*

**Group I:** vehicle; **Group II:** APAP (3g/kg, i.g.); **Group III:** treatment with AE 600 mg/kg, i.g. given in two doses (each of 300 mg/kg) given 12 h before and 1 h after APAP (3g/kg, i.g.), respectively; **Group IV:** treatment with AE 600 mg/kg, i.g. given in a unique dose 1 h after APAP (3g/kg, i.g.).

\* $P < 0.01$  vs Group I.

<sup>§</sup> $P < 0.01$  vs Group II.

<sup>¶</sup> $P < 0.05$  vs Group II.

avoiding the APAP-induced liver peroxidative damage if given only after APAP administration. A similar behaviour was observed when circulating and hepatic levels of protein CG were measured (Tab. 1A and 1B). In fact, APAP induced a significant accumulation, both in blood and in tissue, of protein CG. However, in animals receiving APAP, AE given in two doses (one before and one after APAP administration) completely prevented protein oxidative damage, whereas when the entire dose was given after APAP administration, it showed only a partial protective effect against liver protein CG accumulation without the reduction of plasma protein CG levels.

Finally, APAP treatment produced a significant depletion of hepatic (both proteic and non-proteic) SH pool (Tab. 1B). Only AE given before APAP administration restored the liver pool of NP-SH groups (constituted mainly by GSH) to values measured in controls, although no protective effect was observed concerning P-SH groups (index of the redox state of tissue proteins). These data are in agreement with previous observations showing that anthocyanins can increase GSH expression through the modulation of the Nrf2-ARE pathway (Cimino et al. 2013; Anwar et al. 2014).

Taken together, our findings demonstrate that AE possesses a good protective effect against APAP-induced damage at the level of lipidic cell components, but it is significantly more effective when AE treatment was started before APAP administration. However, different results were obtained with regard to the level of proteins oxidative damage, since, only when given before APAP administration, AE was able to normalise the circulating levels of protein CG. The most common protein modification provoked by oxygen free radicals and reactive oxygen species are carbonyl groups which can arise following oxidative attack on arginine, lysine, threonine and proline residues. Proteins activity and function are strictly dependent on structure and conformation, whose modifications in conditions of oxidative

stress can lead to dysfunction/function loss of proteins and inhibition of their degradation, besides to have also a wide range of downstream functional consequences. Thus, our findings are in accord with the fact that the increased level of protein CG is also correlated to changes in the efficiency of proteolysis and that the half-life of these compounds is quite long, as a function also of defects precisely in their removal. Moreover, AE was unable to normalise the levels of P-SH groups suggesting the irreversibility of the changes induced by APAP at the level of hepatic P-SH groups. However, the levels of hepatic NP-SH groups (of which 95% is represented by GSH) are normalised by AE, although only if given before APAP administration.

### 3. Conclusions

In conclusion, we demonstrated that the administration of an anthocyanin-rich extract is able to protect against liver damage induced by APAP in the rat, as shown by the normalisation of several parameters of hepatic functionality and oxidative stress. However, our data gives the evidence that, to achieve a better protective effect against APAP-induced damage, AE has to be administered, partially at least, before APAP. It is plausible that the hepatoprotective property of this extract is related to a number of different mechanisms, such as free-radical scavenging and antioxidant activities, but also the modulation of expression of cellular antioxidant and cytoprotective genes (Domitrovic 2011; Speciale et al. 2011). These findings could help in the design of future strategies to develop specific nutritional molecules like anthocyanins as agents for the treatment and especially the prevention of hepatotoxicity induced by drugs and in particular by APAP.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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### References

- Ali BH, Mousa HM, El-Mougy S. 2003. The effect of a water extract and anthocyanins of hibiscus sabdariffa L. on paracetamol-induced hepatotoxicity in rats. *Phytother Res.* 17(1):56–59.
- Anwar S, Speciale A, Fratantonio D, Cristani M, Saija A, Virgili F, Cimino F. 2014. Cyanidin-3-O-glucoside modulates intracellular redox status and prevents HIF-1 stabilization in endothelial cells *in vitro* exposed to chronic hypoxia. *Toxicol Lett.* 226(2):206–213.
- Bessemers JG, Vermeulen NP. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Crit Rev Toxicol.* 31(1):55–138.

- Cimino F, Speciale A, Anwar S, Canali R, Ricciardi E, Virgili F, Trombetta D, Saija A. **2013**. Anthocyanins protect human endothelial cells from mild hyperoxia damage through modulation of Nrf2 pathway. *Genes Nutr.* 8(4):391–399.
- Domitrovic R. **2011**. The molecular basis for the pharmacological activity of anthocyanins. *Curr Med Chem.* 18(29):4454–4469.
- Horbowicz M, Kosson R, Grzesiuk A, Debski H. **2008**. Anthocyanins of fruits and vegetables - their occurrence, analysis And role in human nutrition. *Vegetable Crops Research Bulletin.* 68:5–22.
- McGill MR, Jaeschke H. **2013**. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *Pharm Res.* 30(9):2174–2187.
- Speciale A, Chirafisi J, Saija A, Cimino F. **2011**. Nutritional antioxidants and adaptive cell responses: an update. *Curr Mol Med.* 11(9):770–789.
- Wang W, Li J, Wang Z, Gao H, Su L, Xie J, Chen X, Liang H, Wang C, Han Y. **2014**. Oral hepatoprotective ability evaluation of purple sweet potato anthocyanins on acute and chronic chemical liver injuries. *Cell Biochem Biophys.* 69(3):539–548.