

# The state of pro- and antioxidant systems in rats with DMH-induced colon carcinogenesis on the background of extracorporeal detoxification

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## Abstract

**Aim:** Cancer is one of the leading causes of death in the world. The aim of this research was to study the indices of pro- and antioxidant systems in rats with dimethylhydrazine (DMH)-induced colon carcinogenesis on the background of the enterosorbent AUT-M use.

**Materials and methods:** The study was performed on 70 white male rats weighing 200–250 g. Adenocarcinoma of the colon was simulated by subcutaneous injection of the DMH (Sigma-Aldrich Chemie, Japan) at a dose of 7.2 mg/kg once a week during 7 months. Enterosorbent AUT-M was administered intragastrically daily for 21 days after simulation of carcinogenesis at a dose of 1 ml of suspension per 100 g of animal body weight. The state of the pro- and antioxidant systems was studied by the content of oxidative modification of proteins products (OMP), the activity of superoxide dismutase (SOD), catalase (CAT), contents of ceruloplasmin (CP) and reduced glutathione (GSH).

**Results:** It was found that DMH-induced colon carcinogenesis in rats is accompanied by disorders in the antioxidant defense system and activation of free radical oxidation processes. Enterosorbent AUT-M provides a significant reduction in the content of OMP<sub>370</sub> and OMP<sub>430</sub> in both blood serum and liver homogenate of rats. Moreover, the use of enterosorbent AUT-M demonstrated a significant increase in the activity of SOD, CAT, content of GSH and a decrease in CP content in investigated tissues.

**Conclusion:** The use of enterosorbent AUT-M demonstrated prominent potential suppression for oxidative stress and positive effect on antioxidant defense system in rats with DMH-induced colon carcinogenesis.

## Keywords

dimethylhydrazine, colon carcinogenesis, oxidative stress, enterosorbent AUT-M

## Introduction

Cancer is one of the leading causes of death in the world and colorectal cancer is one of the three most commonly diagnosed cancers (Siegel et al. 2020; Sung et al.

2020). Besides an ageing population and dietary habits of high-income countries, unfavourable risk factors such as obesity, lack of physical exercise, smoking and chlamydial infections increase the risk of colorectal cancer Boiko et al. 2019; Borel et al. 2018; Dekker et al. 2019). It is known

that the development of a malignant process is accompanied by multiple pathological manifestations. Cancer cells are highly metabolically active and hypoxic cells, and due to massive growth and insufficient vascular irrigation tend to produce increased reactive oxygen species (ROS) (Sreevalsan and Safe 2013; Arfin et al. 2021). Active generation of ROS enhances the processes of free radical oxidation and leads to the development of oxidative stress (Marushchak et al. 2016, 2019). Uncontrolled generation of free radicals provokes a cascade of reactions that negatively affect crucial biomolecules: genomic DNA, lipids and proteins (Posokhova et al. 2018). It should be noted that molecules altered as a result of impact by the ROS can be viewed as signals that carry the biological information required for regulation of various cellular functions, in part, for initiation of apoptosis (Marushchak et al. 2017a, 2017b). Therefore, influence of enterosorption on the development of oxidative stress in cancer patients has exceptional importance (Shevchuk et al. 2012).

On the other hand, one of the main methods in the treatment of cancer is chemotherapy. However, toxic side effects of the components of chemotherapy can also cause oxidative stress, reduced intracellular redox potential, which can lead to discontinuation of treatment before a clear antitumor effect. A special place in this problem solving is occupied by the methods of sorption therapy the possibilities of which are being constantly expanded due to the development of new effective sorbents (Sakhno et al. 2014). In the literature there are data indicating a strong adsorption potential of sorbents with a macroporous structure (Shevchuk et al. 2012; Sakhno et al. 2013, 2014; Kachur et al. 2020), such as AUT-M (activated carbon tissue material), which consists of carbon fibers and has a specific pore surface area of about 2000–2500 m<sup>2</sup>/g.

The aim of the research was to study the indices of pro- and antioxidant systems in rats with dimethylhydrazine-induced colon carcinogenesis on the background of the enterosorbent AUT-M use.

## Materials and methods

The study was performed on 70 white male rats weighing 200–250 g. Laboratory animals were kept on a standard diet of the vivarium of I. Horbachevsky Ternopil National Medical University in compliance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Research and Other scientific goals. The study was approved by the Ethical Committee of I. Horbachevsky Ternopil National Medical University (Excerpts from Minutes №61, dated 13.11.2020).

Experimental study design comprised three groups: I - control animals, which received saline subcutaneously into the interscapular area once a week for 30 weeks; II - animals with simulated adenocarcinoma of the colon; III - animals with simulated adenocarcinoma of the colon and 21-day extracorporeal detoxification with the sorbent AUT-M.

Adenocarcinoma of the colon in rats was simulated by subcutaneous injection of the carcinogen 1,2 dimethylhydrazine (DMH) (Sigma-Aldrich Chemie, Japan), pre-diluted with saline into the interscapular area at a dose of 7.2 mg/kg (Deryagina et al. 2009). Subcutaneously injected DMH is sequentially metabolized to methylazoxy-methanol in the liver. This metabolite is transported to the colon via the bile or blood circulation to cause DNA mutations from G:C to A:T in genes involved in cell proliferation (Jikihara et al. 2015). DMH was injected once a week during 7 months. After modeling the cancer process, the animals underwent detoxification correction with the sorbent AUT-M. The drug was administered intragastrically daily for 21 days. The daily dose of sorbent was 1 ml of suspension (corresponding to 0.2 g of net weight of the drug) per 100 g of animal body weight.

The rats were euthanized under deep thiopental-sodium anesthesia by cardiac puncture once per month for 7 months, and on the 14<sup>th</sup> and 21<sup>st</sup> day of the administration of the enterosorbent AUT-M.

Blood and liver samples were used for further investigations. To obtain blood serum, blood samples were allowed to clot (at room temperature for 30 min), then they were centrifuged for 15 min at 1200 g and room temperature. To prepare 10% homogenate, liver samples, taken immediately after euthanasia, were cooled to 1–3 °C in saline, dried with filter paper and homogenized in 0.05 M Tris-HCl buffer (pH 7.4) using a magnetic homogenizer SilentCrusher S, (Heidolph, Germany).

Prooxidant system was evaluated in blood serum and liver homogenate by the content of oxidative modification of proteins products (OMP) - aldehyde- and ketone derivatives of a neutral character (OMP<sub>370</sub>) and aldehyde- and ketone derivatives of an alkaline character (OMP<sub>430</sub>). Antioxidant system was evaluated by the activities of superoxide dismutase (SOD), catalase (CAT), content of ceruloplasmin (CP) and reduced glutathione (GSH) (Vlizlo et al. 2012).

Statistical data analysis has been performed using STATISTICA 13 (TIBCO Software Inc., 2018). Parametric and nonparametric methods of evaluation of the obtained data were used for statistical processing of the results. For all indices, the arithmetic mean of the sample (M) and the error of the arithmetic mean (m) were calculated. The reliability of the difference between the values between the independent quantitative values was determined by the normal distribution by the Student's t test, in other cases - by the Mann-Whitney test. The difference between the values was considered probable at p<0.05.

## Results

It was found that in animals with DMH-induced colon carcinogenesis at 1<sup>st</sup> month of the experiment in the blood serum and liver homogenate, the content of OMP<sub>370</sub> increased by 2.3 times (p<0.05) and by 1.4 times (p<0.05), respectively vs control animals. At the end of the simula-

tion of adenocarcinoma of the colon (at 7<sup>th</sup> month), this index increased in blood serum by 5.4 times ( $p < 0.05$ ), in the liver homogenate – by 2.7 times ( $p < 0.05$ ) compared with non-carcinogenic animals (Table 1).

Similar dynamics of aldehyde- and ketone derivatives of an alkaline character ( $OMP_{430}$ ) changing was found in blood serum and liver homogenate of rats during all experiment: at 1<sup>st</sup> month in blood serum this index significantly increased by 1.3 times, in the liver homogenate – by 1.5 times vs control group. At 7<sup>th</sup> month of the experiment, the content of  $OMP_{430}$  in blood serum increased by 2.9 times, in the liver homogenate – by 2.4 times ( $p < 0.05$ ) compared with control group.

We have found that the use of the enterosorbent AUT-M provides a significant ( $p < 0.05$ ) reduction in the content of  $OMP_{370}$  and  $OMP_{430}$  in both blood serum and liver homogenate of animals with DMH-induced colon carcinogenesis. The content of  $OMP_{370}$  on the 21<sup>st</sup> day of the study decreased by 200.0% and 83.0%, respectively; the content of  $OMP_{430}$  decreased by 100.0% and 55.0%, respectively compared with the affected animals without use of enterosorbent (Figure 1).

SOD and CAT serve as the first line of antioxidant defense, being the scavengers of ROS. We found that in rats with DMH-induced colon carcinogenesis, statistically significant decreasing of SOD activity in the liver homogenate was starting from 4<sup>th</sup> month of the affection (by 1.4 times vs control group) (Table 2). This index decreased in the following terms of the experiment (at 7<sup>th</sup> month SOD activity was 1.75 times lower in rats with DMG-induced colon carcinogenesis vs control group).

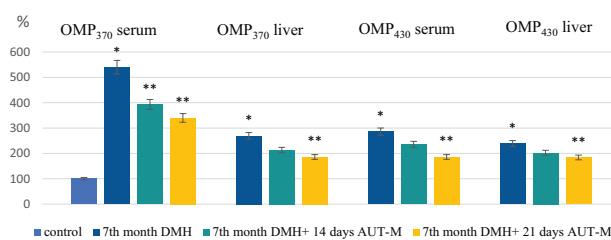
Along with SOD, the dismutation reaction is catalyzed by a copper-containing protein with enzymatic activity – CP. In the group of rats with DMH-induced colon carcinogenesis, we determined a significant ( $p < 0.05$ ) increase in the content of CP in blood serum vs control group (by 1.4 times at 1<sup>st</sup> month of the experiment). After 5 months of the colon adenocarcinoma modeling, this index increased by 5.6 times ( $p < 0.05$ ), after 30 weeks of the colon adenocarcinoma modeling, we found an increase in the content of CP by 3.8 times ( $p < 0.05$ ) vs control animals (Table 2).

Analyzing changes in CAT activity we found that in the blood serum of rats with DMH-induced colon carcinogenesis this index significantly decreased by 1.6 times at 3<sup>rd</sup> month of the experiment and continued to

**Table 1.** The content of OMP products in blood serum and liver homogenate of rats in the dynamics of DMH-induced colon carcinogenesis, ( $M \pm m$ ).

Index/Group of animals, period of affection	$OMP_{370}$ mmol/g protein		$OMP_{430}$ mmol/g protein	
	Blood serum	Liver homogenate	Blood serum	Liver homogenate
Control, n=7	0.15±0.01	0.29±0.02	0.30±0.02	0.38±0.02
1 <sup>st</sup> month, n=7	0.34±0.02*	0.41±0.02*	0.39±0.02*	0.56±0.04*
2 <sup>nd</sup> month, n=7	0.47±0.03*	0.47±0.03*	0.49±0.02*	0.64±0.04*
3 <sup>rd</sup> month, n=7	0.51±0.04*	0.60±0.04*	0.57±0.04*	0.68±0.04*
4 <sup>th</sup> month, n=7	0.59±0.04*	0.68±0.04*	0.64±0.05*	0.73±0.05*
5 <sup>th</sup> month, n=7	0.68±0.05*	0.70±0.05*	0.74±0.05*	0.80±0.06*
6 <sup>th</sup> month, n=7	0.74±0.05*	0.72±0.06*	0.80±0.05*	0.83±0.06*
7 <sup>th</sup> month, n=7	0.81±0.06*	0.78±0.06*	0.86±0.06*	0.91±0.08*

Note: \* - significant differences in comparison to control animals.



**Figure 1.** Dynamics of the content of OMP products in the blood serum and liver homogenate of the rats with adenocarcinoma of the colon on the background of the enterosorbent AUT-M use. Note: \* – significant differences in comparison to control animals; \*\* – significant differences between the indices of carcinogenic animals and carcinogenic animals that received enterosorbent.

decrease during the following months of the experiment (at 7<sup>th</sup> month CAT activity was 3.2 times lower in rats with DMH-induced colon carcinogenesis vs control group) (Table 2).

GSH also is one of the central components of antioxidant defense system. It non-enzymatically inactivates hydrogen peroxide and inhibits ROS generation (Shelly 2013). We found an increasing of the GSH content in blood serum of rats with DMH-induced colon carcinogenesis (at 3<sup>rd</sup> month of the experiment – by 1.6 times ( $p < 0.05$ )) vs control group (Table 2). In the following terms of the experiment, the content of GSH in the blood serum of rats with DMH-induced colon carcinogenesis significantly decreased (at 7<sup>th</sup> month content of GSH was

**Table 2.** Indices of the antioxidant system in blood serum and liver homogenate of rats in the dynamics of DMH-induced colon carcinogenesis ( $M \pm m$ ).

Index / Group of animals, period of affection	SOD in liver homogenate, (units/mg)	CP in blood serum, (mg/l)	CAT in blood serum, ( $\mu$ cat/l)	GSH in blood serum, (mmol/l)
Control, n=7	0.42±0.03	1.99±0.22	1.29±0.66	1.21±0.03
1 <sup>st</sup> month, n=7	0.39±0.03	2.81±0.26*	1.19±0.07	1.35±0.04*
2 <sup>nd</sup> month, n=7	0.36±0.03	3.32±0.30*	1.16±0.06	1.42±0.06*
3 <sup>rd</sup> month, n=7	0.32±0.03	5.25±0.40*	0.80±0.05*	1.94±0.06*
4 <sup>th</sup> month, n=7	0.29±0.02*	7.07±0.54*	0.64±0.04*	1.04±0.05*
5 <sup>th</sup> month, n=7	0.25±0.02*	11.2±0.46*	0.55±0.04*	0.77±0.06*
6 <sup>th</sup> month, n=7	0.23±0.02*	7.71±0.52*	0.49±0.04*	0.72±0.04*
7 <sup>th</sup> month, n=7	0.24±0.01*	7.58±0.66*	0.40±0.03*	0.71±0.04*

Note: \* - significant differences in comparison to control animals.

**Table 3.** Indices of the antioxidant system in blood serum and liver homogenate of rats with DMH-induced colon carcinogenesis on the background of the enterosorbent AUT-M use ( $M \pm m$ ).

Index /Group of animals, period of affection	SOD in liver homogenate, (units/mg)	CP in blood serum, (mg/l)	CAT in blood serum, (mcat/l)	GSH in blood serum, mmol/l
Control, n=7	0.42±0.03	1.99±0.22	1.29±0.66	1.40±0.03
7 <sup>th</sup> month DMH, n=7	0.24±0.01*	7.58±0.66*	0.40±0.03*	0.79±0.05*
7 <sup>th</sup> month DMH+AUT-M (14 days), n=7	0.34±0.03**	5.72±0.27**	0.54±0.03**	1.04±0.03**
7 <sup>th</sup> month DMH+AUT-M (21 days), n=7	0.36±0.04**	5.42±0.31**	0.63±0.04**	1.15±0.04**

Note: \* – significant differences in comparison to control animals; \*\* – significant differences between the indices of carcinogenic animals and carcinogenic animals that received enterosorbent.

1.7 times ( $p < 0.05$ ) lower in rats with DMH-induced colon carcinogenesis vs control group)

The use of enterosorbent AUT-M had a positive effect on the state of enzymatic and non-enzymatic units of antioxidant defense system (Table 3). Thus, SOD activity in the liver homogenate significantly ( $p < 0.05$ ) increased on the 14<sup>th</sup> day of enterosorbent use by 1.4 times, on the 21<sup>st</sup> day – by 1.5 times vs group of affected animals without use of enterosorbent.

Analyzing changes in the content of CP in the blood serum of rats we found the decreasing of this index after the use of enterosorbent AUT-M. On the 14<sup>th</sup> day of enterosorbent use – by 1.3 times ( $p < 0.05$ ), on the 21<sup>st</sup> day – by 1.4 times ( $p < 0.05$ ) vs group of affected animals without use of enterosorbent. The use of sorbent AUT-M helps to restore catalase activity. We have found that on the 14<sup>th</sup> day of enterosorbent use catalase activity in the blood serum significantly increased by 1.3 times, on the 21<sup>st</sup> day – by 1.6 times ( $p < 0.05$ ) vs group of affected animals without use of enterosorbent. Analyzing changes in the content of GSH in the blood serum of rats we found the increasing of this index after the use of enterosorbent AUT-M: on the 14<sup>th</sup> day of enterosorbent use – by 1.3 times ( $p < 0.05$ ), on the 21<sup>st</sup> day – by 1.5 times ( $p < 0.05$ ) vs group of affected animals without use of enterosorbent.

## Discussion

According to the results of our studies, it was found that in blood serum and liver homogenate of rats with DMH-induced colon carcinogenesis the content of aldehyde- and ketone derivatives of a neutral character ( $OMP_{370}$ ) progressively increased during all terms of the experiment vs control animals. Similar dynamics of aldehyde- and ketone derivatives of an alkaline character ( $OMP_{430}$ ) changing was found in blood serum and liver homogenate of rats with DMH-induced colon carcinogenesis with the maximum increasing at 7<sup>th</sup> month of the experiment. This indicates hyperproduction of ROS and a progressive increase in the activity of free radical processes in case of DMH-induced colon carcinogenesis. Some protein oxidative modifications promote loss of protein function, cleavage or aggregation, and some result in proteo-toxicity and cellular homeostasis disruption (Demasi et al. 2021). In addition, the negative effect of protein oxidative modifications is associated with the fact that they are a source of free ra-

dicals and deplete the supply of cellular antioxidants (Ray et al. 2012; Marushchak et al. 2019).

SOD is the first detoxification enzyme and most powerful antioxidant in the cell. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against ROS. It catalyzes the dismutation of two molecules of superoxide anion to hydrogen peroxide and molecular oxygen, consequently rendering the potentially harmful superoxide anion less hazardous (Ighodaro et al. 2018). We have found a significant decrease in SOD activity in the liver homogenate, starting from the fourth month of DMH-induced carcinogenesis modeling. The decrease in the activity of SOD may be due to the fact that ROS directly affect the degree of oxidation of metal ions in the active centers of enzymes, which leads to inhibition of their functioning. Another reason for the decrease in SOD activity may be the accumulation of hydrogen peroxide, which is an inhibitor of the enzyme (Perse 2013).

Along with SOD, the dismutation reaction is catalyzed by a copper-containing protein with enzymatic activity – CP. In the dynamics of the colon adenocarcinoma development in rats, an increase in CP activity was observed. Obviously, this may be due to a change in its catabolism in the affected organism. Normally, the catabolism of CP occurs in the liver with neuraminidase, which performs desialization to asialoceruloplasmin, which can be excreted from the body. In affected hepatocytes, desialization is probably less effective, which is why the breakdown of CP in the liver is inhibited, which leads to an increase in its content in the blood serum (Perse 2013).

CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen. The enzyme uses either iron or manganese as a cofactor and catalyzes the degradation or reduction of hydrogen peroxide to water and molecular oxygen, consequently completing the detoxification process initiated by SOD (Ighodaro et al. 2018). We found that the catalase activity in the blood serum of the affected rats significantly decreased by the third month of the experiment and continued to decrease during the following terms of the experiment. In addition to severe hyperproduction of ROS, one of the reasons for the decrease in catalase activity in DMH-induced carcinogenesis, may be the suppression of protein-synthesizing function of the liver under the action of toxins (Perse 2013).

GSH is one of the most abundant non-protein thiols, which is also involved in the antioxidant defense system,

including protecting proteins during an oxidative stress through the reversible glutathionylation of active thiols and maintaining of the reduced form of multiple antioxidant enzymes through the process of redox cycling, which involves repeated reduction–oxidation (SH to SS) reactions (Sreekumar et al. 2021). We found that the content of GSH in the blood serum of rats with DMH-induced colon carcinogenesis progressively decreased starting from the fourth month of the experiment.

Our results indicate that along with the simulated oncological process, animals develop disorders in the antioxidant system combined with activation of free radical oxidation processes. The use of enterosorbent AUT-M demonstrated prominent potential suppression for oxidative stress in rats with DMH-induced colon carcinogenesis. We found that enterosorbent AUT-M provides a significant reduction in the content of OMP<sub>370</sub> and OMP<sub>430</sub> in both blood serum and liver homogenate of rats. Moreover, the use of enterosorbent AUT-M demonstrated positive effect on antioxidant defense. There was a significant increase in the activity of SOD, CAT, and a decrease in CP content in investigated tissues. The use of enterosorbent AUT-M also helped to restore the functional activity of the glutathione system, by increasing content of GSH in the blood serum of rats with DMH-induced colon carcinogenesis.

Our results are consistent with the data of other researchers who demonstrated that oral carbon adsorbents have capability to repair ROS-induced damage

(Nikolaev et al. 2011; Shevchuk et al. 2019). The main mechanisms of action of enterosorption, which explain their restorative effects, are the adsorption of different exogenous and endogenous toxins, among them the products of lipid and protein peroxidation (Shevchuk et al. 2019). Elimination of various exogenous and endogenous toxins, including lipid and protein peroxidation products, can occur by osmosis or diffusion through the capillary walls of the villi of the small intestine, followed by fixation on the sorbent (Nikolaev et al. 2011; Mikhalovsky et al. 2012).

## Conclusion

DMH-induced colon carcinogenesis in rats is accompanied by disorders in the antioxidant defense system (a significant decrease in the activity of SOD, CAT and content of GSH) and activation of free radical oxidation processes (a significant increase of OMP) in blood serum and liver homogenate. The use of enterosorbent AUT-M demonstrated prominent potential suppression for oxidative stress and positive effect on antioxidant defense system in rats with DMH-induced colon carcinogenesis. The obtained results can serve as a basis for further study of the possibility of enteral sorption therapy use in patients with colorectal cancer in order to reduce the manifestations of chemotherapy side effects and facilitate the course of the disease.

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