THE CCl_4 ACTION UPON PHYSIOLOGICAL INDICES IN *Lepus timidus* AND THE PROTECTIVE ROLE OF SOME SUBSTANCES

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Abstract. Aim of this study is to demonstrate the hepatoprotective role of grape seed oil and *Cynara scolymus* leaf extract. This experiment lasted for 12 days performed on male and female rabbit. The animals were intoxicated in the latest day of experiment (day 12^{th}) with CCl_4 in a dose of 30µl/100g body weight and a group of them was treated for 12 days with 0.4 mg/kg body weight/day of *Cynara scolymus* (artichoke) leaf extract while another group was treated with grape seed oil (1ml/kg body weight/day). Intoxication with CCl_4 caused an increase a glycemia and a number of leukocytes, decrease cholesterol, triglycerides value, and a number of erythrocytes. We observed that the extract of *Cynara scolymus* leaf and the grape seed oil had a protective effect against CCl_4 intoxication.

Keywords: *Cynara scolymus*, grape seed oil, glycemia, triglycerides, cholesterol, erythrocytes and leukocytes

INTRODUCTION

Research on the action of harmful agents on various physiological indices at homeothermes are quite numerous and necessary for the development of biotechnology in animal growth and their recovery for economic interest. We underline the concerns of researchers to find antidote compounds to the toxic action of various chemical pollutants [1, 3, 10, 14, 15], to which we to add our own research.

Liver diseases constitute a major problem of world wide proportions. Carbon tetrachloride (CCl_4) is a well known hepatotoxin that is widely used to induce acutetoxic liver injury in a large range of laboratory animals [2, 13].

Artichoke leaf extract has been shown to be a natural remedy which improves various digestive health disorders, shown to significantly lowered blood cholesterol levels, prevent heart disease and atherosclerosis - plaque problems, enhance detoxification reactions, as well as protect the liver from damage. This combination of benefits is very important to healthy liver function. During detoxification of the liver, the toxic substance is often initially converted to an even more toxic form. Without adequate protection, every time the liver neutralizes a toxin, it is damaged in this process.

Among other beneficial effects, the active compounds in grape seed are believed to have antioxidant properties. Antioxidants are substances that destroy free radicals - damaging compounds in the body that alter cell membranes, tamper with DNA (genetic material), and even cause cell death. Free radicals occur naturally in the body, but environmental toxins (including ultraviolet light, radiation, smoke, certain prescription and non-prescription drugs, and air pollution) can also increase the number of these damaging particles. Antioxidants found in grape seeds can neutralize free radicals and may reduce or even help prevent some of the damage they cause.

The purpose of this study was to investigate possible beneficial effects of grape seed oil and *Cynara scolymus* leaf extract on CCl_4-induced acute hepatotoxicity in rabbit.

MATERIALS AND METHODS

Our researches have been carried out on domestic rabbits (*Lepus timidus*) aged five months, both males and females, having an average weight of 1850±100g. The animals were divided into four lots: the lot including the control animal, which was made up of five females and five males, and an experiment lots (lot I, lot II and lot III) including the same number of specimens: lot I includes 10 animals, male and female, intoxicated with CCl_4 in a dose of 30µl/100g body weight in the latest day of experiment (day 12^{th}); lot II includes 10 animals, male and female, treated for 12 days with 0.4mg/kg body weight/day extract of *Cynara scolymus* (artichoke) leaf (preventive treatment) and intoxicated with CCl_4 in a dose of 30µl/100g body weight in the latest day of experiment (day 12^{th}); lot III includes 10 animals, male and female, treated for 12 days with 1ml/kg body weight/day grape seed oil (preventive treatment) and intoxicated with CCl_4 in a dose of 30µl/100g body weight in the latest day of experiment (day 12^{th}).

The lots were kept under the same kind of conditions, the animals receiving, on a daily basis, a sufficient quantity of food and water.The administration of the toxic product being done through gastric gavages. The determinations of the physiological indices (glycemia, cholesterol and triglycerides value, a number of erythrocytes and leukocytes) were conducted after the administration of the toxic substance (CCl_4). The determination of the amount of glucose in the animals' blood was made by using two different techniques: the colorimetric dosage with o-toluidine, and by means of the *Glucotrend* glucometer; cholesterol and triglycerides level has been determinate using an Accutrend GCT. The number of erythrocytes and leukocytes was microscopically determined with a Thoma cells numbering chamber, by using a small amount of blood collected from the marginal vein of the ear [11].

RESULTS

Glucose values recorded in all four groups of animals are shown in Fig. 1. The analysis of this graph

shows an increase of the blood sugar in animals receiving CCl_4 in the amount of 30 µl / 100g body weight by 197.17% to control value. The other two lots of animals to which the toxic dose was administered 12 days after treatment with *Cynara scolymus* leaf extract (lot II) and grape seed oil (lot III), show a decrease in the value of this index but this is insignificant.

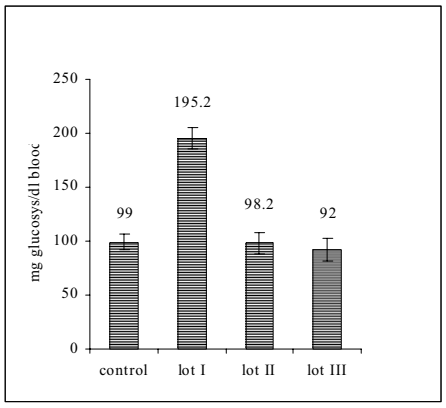

Figure 1. The average values of glycemia in *Lepus timidus* while treated with CCl_4.

Fig. 2 shows cholesterol values recorded for the three groups. The analysis of this graph shows a decrease of cholesterol in animals treated with CCl_4 by 27% as compared to control value. The use of preventive treatment with *Cynara scolymus* leaf extract, respectively grape seed oil, counteracts the toxic effect of CCl_4, the cholesterol value in these animals being similar to control value.

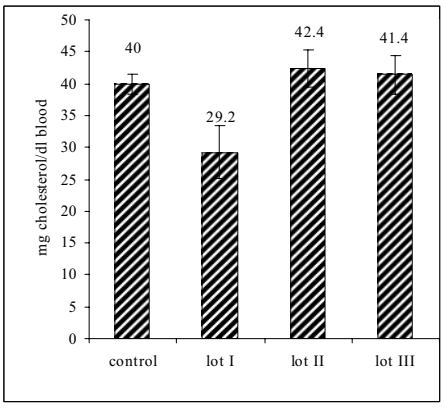

Figure 2. The average values of cholesterol in *Lepus timidus* while treated with CCl_4.

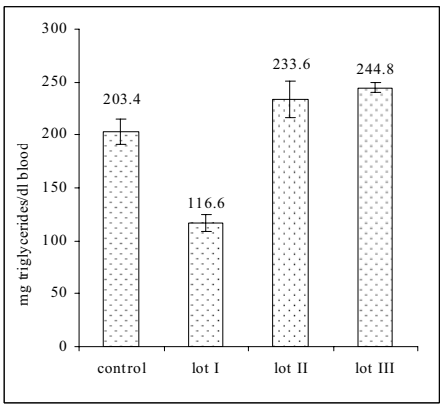

Figure 3. The average values of triglycerides in *Lepus timidus* while treated with CCl_4.

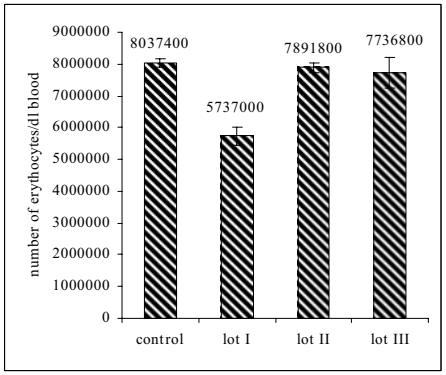

Figure 4. The average values of number of erythrocytes in *Lepus timidus* while treated with CCl_4

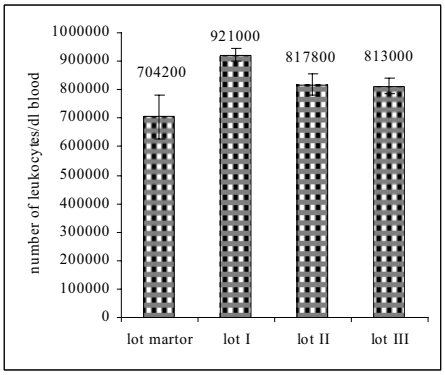

Figure 5. The average values of number of leukocytes in *Lepus timidus* while treated with CCl_4.

The triglyceride levels (Fig. 3) show a decrease in the value of this index for animals receiving CCl_4 in the amount of 30µl/100g body weight by 42.67% as compared to control value. An increase by 14.84% compared to control value of triglycerides is obtained for animals treated with a dose of CCl_4 in the amount of 30µl/100g body weight on the basis of treatment with artichoke leaf extract for 12 days. There is an increase in the value of this index by 20.35% as compared to control value in case of animals treated with a dose of CCl_4 in the amount of 30µl/100g body weight on the basis of treatment with grape seed oil.

Fig. 4 shows the number of erythrocytes. The analysis of these figures shows a decrease in the number of erythrocytes in all experimental variants. Thus, a dose of CCl_4 in the amount of 30µl/100g body

weight decreases by 28.62% as compared to control value. Administering the same dose of toxic substance on the basis of preventive treatment with artichoke leaf extract, results in a decrease of 1.81%, the decrease is insignificant for animals treated with grape seed oil receiving a single dose of CCl_4 in the amount of 30µl/100g body weight.

CCl_4 intoxication causes an increase in white blood cells (Fig. 5) (by 30.78% as compared to control value), while preventive treatment with *Cynara scolymus* leaf extract or grape seed oil for 12 days, significantly reduce toxicity, the index values recorded in animals (lot II, III) maintaining within normal limits.

DISCUSSIONS

Manifestation of toxic effect of CCl_4 by increasing blood glucose levels can be explained by toxicity of glycolysis enzyme systems and other metabolic pathways of glucose utilization at the cellular level. Similar results were obtained by other researchers [14, 15], who noticed that CCl_4 causes steatosis, necrosis, inflammation and liver fibrosis. The authors cited have noticed the emergence of massive lesions in the liver but reduced in kidney in rats treated with CCl_4.

Values close to normal blood sugar levels in the other 2 groups of animals suggest that *Cynara scolymus* leaf extract, which has a rich content of insulin, as well as the grape seed oil, exercise a strong protective action against harmful effect of the toxic.

Similar research was conducted by Pintea [12] on Wistar rats showing that hepatotoxic CCl_4 acts by a mechanism of lipid peroxidation in the membrane. He also noticed a decrease in cholesterol and triglycerides level at the expense of phospholipids, unsaturated fatty acid content decreased at the expense of saturated fatty acids, serum transaminase levels increased, reduced glutathione levels, cytochrome P450 and he corresponding enzymes decreased.

The fact that our experiments recorded decreases in concentrations of cholesterol and triglycerides may be explained by the fact that the toxic effect of CCl_4 is diminished by daily administration of *Cynara scolymus* leaf extract having hepatoprotective effects. The antitoxic action of grape seed oil is likely to be weaker the value which caused increased value of triglycerides. Our results are similar to those obtained by Pintea [12] on rats, which showed that *Calendula* oil protects liver cells against toxic action of carbon tetrachloride. As a consequence of lipids peroxidation under the action of carbon tetrachloride, the liver tissue undergoes a change of composition ratio of triglycerides and phospholipids. Since unsaturated fatty acids enter the composition of phospholipids, they are more affected by the action of carbon tetrachloride, which is manifested by increasing the quantity of triglycerides as compared to phospholipids. Decrease in the amount of phospholipids is visible in the microsomal fraction, which is accompanied by a significant increase in triglyceride content [7]. The decreased number of red blood cells followed by an increased number of leukocytes in CCl_4 intoxication is possibly due to a state of anemia.

Similar results were obtained by Păunescu [9] in studies on rabbits receiving solanine obtained from berries of *Solanum dulcamara.* In the lake frog experimental intoxication with CCl_4, Păunescu [10] found a decrease in the number of erythrocytes and a return value of this index against a preventive treatment with thiourea.

Administration of rats with an aqueous extract of *Phyllanthus* prior to CCl4 administration showed significant protection on the carbon tetrachloride induced mitochondrial dysfunction [8]. In other studies, morin prevents acute liver damage in CCl_4 intoxication in rats by inhibiting the production of TNF-α, IL-1β, IL-6, and iNOS [4]. Tender coconut water (TCW) has an hepatoprotective and antioxidant effect in carbon tetrachloride intoxicated female rats [5]. Hepatoprotective effect is evidenced from the histopathological studies of liver wich did not show any fatty infiltration or necrosis as observed in CCl_4-intoxicated rats.

The hepatoprotective effects of colchicines on acute liver injury induced by carbon tetrachloride or by dimethylnitrosamine in mice were examined by the Mizuoka [6]. Administration of colchicines 18 h before hepatotoxins markedly suppressed liver injury, whereas colchicines administration 2 h before the hepatotoxins accelerated it. The hepatoprotective effect evoked by colchicines was due to reduction in liver cytochrome P450 content and P450 2E1 activity.

Experimental intoxication of rabbits with CCl_4 causes an increase in circulating blood glucose and leucocytes, decreased cholesterol, triglycerides and number of erythrocytes. Preventive treatment with *Cynara scolymus* leaf extract, and grape seed oil for 12 days, counteracts the toxic effect of carbon tetrachloride, the values of the studied physiological indices (blood sugar, cholesterol, triglycerides, number of erythrocytes and leukocytes) maintaining within normal limits. The grape seed oil is considered the best medicine - food to lower and maintain the normal rate of cholesterol. The hepatoprotective effects of artichoke leaf extract (*Cynara scolymus*) are also widely accepted.

REFERENCES

[1] Anupam, B., Alok, S., Malay, C., (1995): Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. Journal of Ethnopharmacology, vol. 47(2): 69-74.

[2] Coprean, D., Şchiopu, S., Pintilie, G., Zamfirescu, S., (2002): Tratamentul cu ueil de *Oenothera biennis* are efect hipolipemiant şi hipocolesterolemiant la şobolanul Wistar. An.Of.Nat.Cell.Biol.Soc., 7(1): 361-368.

[3] İlhan, N., Seçkin, D., (2005): Protective effect of *Nigella sativa* seed on CCl_4-induced hepatotoxicity. F.Ü. Sağlık Bil. Dergisi, 19(3): 175-179.

[4] Lee, H.S., Jung, K.H., Hong, S.W., Park, I.S., Lee1, C., Han, H.K., Lee, D.H., Hong, S.S., (2008): Morin protects acute liver damage by carbon tetrachloride (CCl_4) in rat. Arch. Pharm. Res, vol. 31, no.9, pp. 1160-1165.

[5] Loki, A.L., Rajamohan, T., (2003): Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats. Indian Journal of Biochemistry&Biophysics, vol. 40, pp. 354-357.

[6] Mizuoka, H., Shikata, N., Yang, J., Takasu, M., Inoue, K., Tsubura, A., (1999): Biphasic effect of colchicine on acute liver injury induced by carbon tetrachloride or by dimethylnitrosamine in mice. J. Hepatol, Vol.31, pp. 825-833.

[7] Ohkawa, H., Ohishi, N., Yagi, K., (1979): Assay for lipid peroxides in animal tissues. Analytical Biochemistry, 95: 351-358.

[8] Padma, P., Setty, O.H., (1999): Protective effect of *Phyllanthus* against CCl_4-induced mitochondrial disfunction. Life Science, vol.64, pp. 2411-2417.

[9] Păunescu, A., Ponepal, C.M., Drăghici, O., Marinescu, Al.G., (2009): Changes of some functional parameters in domestic rabbit (*Lepus timidus*) under the influence of infusion of *Solanum dulcamara* baccae. Proceedings of the Second International Conference, Lozenec, Bulgaria, pp. 1-3.

[10] Păunescu, A., Ponepal, C.M., Brînzea, G., (2005): The CCl_4 action upon physioplogical indeces in *Rana ridibunda* and the protective role of thioureea. 5th International Conference of phD Students, University of Miskolc, Hungary, pp. 367-372.

[11] Picoş, C.A., Năstăsescu, Gh., (1988): Lucrări practice de fiziologie animală. University of Bucharest Press, pp. 122-123, 192-195.

[12] Pintea, A., (2002): Caracterizarea şi testarea uleiului polinesaturat din seminţe de *Calendula officinalis* L.The final report of research contract type A.

[13] Rao, P.S., Mangipudy, R.S., Mehendale, H.M., (1997). Tissue injury and repair as parallel and opposing responses to CCl_4 hepatotoxicity: a novel dose-response. Toxicology, 118: 181-193.

[14] Rusu, M., Borşa, M., Roman, I., Puică, C., (2002): Implicarea radicalului liber CCl^{3-} în modificarea activităţii enzimatice la nivelul unor organe metabolice şi imuno-endocrine. An.Of.Nat.Cell.Biol.Soc., 7(1): 257-261.

[15] Şchiopu, S., Coprean, D., Moisă, G.I., Zamfirescu, S., (2002): Studiul efectului hepatoprotector al extractului de *Artemisia vulgaris* în intoxicaţia acută cu CCl_4 la şobolanul Wistar. An.Of.Nat.Cell.Biol.Soc., 7(1): 369-375.