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THE EFFECT OF *VACCINIUM VITIS IDAEA* L. EXTRACT ADMINISTRATION ON KIDNEY STRUCTURE AND FUNCTION IN ALCOHOL INTOXICATED RATS

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ABSTRACT: The study was focused on the assessment of the protector potential of 1:1 hydro-alcoholic extract of *Vaccinium vits idaea* L (lingonberry) (in a dose of 200 mg/100 g bw d.s.) On some kidney morphological aspects and biochemical blood parameters (urea and creatinine) relating to impairment of kidney function in white Wistar rats due to a subchronic alcohol intoxication.Extracts were administered for 15 days in white female Wistar rats, weighing 150 ± 10 g, or at the same time with alcohol 50^0 (6g/kg body). We found that single administration of vegetable extract of lingonberry not caused toxic effects on the kidney function, while treating animals with lingonberry extract had beneficial effects in preventing and protecting parameters of kidney function taken into study (urea and creatinine), in terms of alcoholic intoxication.

Keywords: alcohol intoxication, lingonberry extract, rats, kidney

INTRODUCTION:

Phytotherapy supposes utilization of some medicinal plants in curative or/and prophylactic goals. It uses plants under total or partial extracts form in contrast with allopathic medicine which recommend some substances extracted from plants.

Many xenobiotics like: alcohol ethylic, some drugs and pesticides, ccl_4, nitrosamines, organic solvents, etc, with whom the body came in contact by destabilization, produce free radicals which interfere with the main metabolically processes (Rusu *et al.*, 2005, 2007).

The presence of noxious effects of some free radicals have imposed finding of some remedies. Between these remedies also occur some extracts or biopreparates from plants (Weiss & Fintelmann, 2000). Thanks to its pharmaceutical properties we have chosen to study a hidroalcoholic extract of lingonberry.

Lingonberry (*Vaccinium vitis-idaea* L.) is a small, evergreen wild shrub, with edible red berries. It has creeping, spreading shoots and typically grows in low, dense mats (Trehane, 2004; Taylor & Douglas, 1995; Petrides, 1972) and is spread across northern latitude areas. In Romanian flora, the Ericacee are represented by 14 species of dwarf shrubs and subshrubs, spread in the mountainous area where it forms characteristic associations (Anghel *et al.*, 1965; Ghişa *et al.*, 1970). During recent years, the american blueberry (*Vaccinium corymbosum* L.) Was introduced in culture, for the production of fruit (Eck & Childers, 1992; Popescu *et al.*, 1992).

The least-studied species of these is the lingonberry (*Vaccinium vitis-idaea* L.). If the lingonberry leaves (*Vitis idaeae folium*) are chemical and pharmacological well characterised, the confusion is on its fruit, it extrapolates the action of leaves on them, too.

Compared with the lingonberry leaves, fruits have been less studied. The fruits contain anthocyanins, giving the red color at maturity of these fruits (Li *et al*. 2011). They also contain proantocians, organic acids, flavonoids, vitamins and mineral salts (Ciulei *et al*. , 1993; Istudor, 1998). The presence of arbutoside in fruits haven't been signaled so far, but only the one of benzoic acid 55-310 mg/100 g in fresh and dried fruits (Tamas *et al.*, 2012). Therefore, arbutoside is only present in the leaves of lingonberry.

Benzoic acid from the fruit is not found in the free state but bound in the form of vacciniin, the benzoic compound of glucose and may be issued only after acid hydrolysis. It follows that the disinfectant and antifungal urinary tract actions of lingonberry fruit would be due to benzoic acid released with the digestive enzymes help.

Diseases preventable by lingonberry: due to northern areas where it is spread, the extremely harsh climate that did not allow the growth and consumption of other plants, the populations of those places could prove as exceptional preventive capacities of some of the most diverse diseases: urinary infections (Howell, 2002); kidney stones; diabetes; atherosclerosis; cancer illness due to carotenoids (β-carotene) (Hannum, 2004); respiratory virus infection-laboratory studies have revealed a strong antiviral action of lingonberry leaves, which block the virus replication. Also, animal studies have shown how the lingonberry can lower inflammatory molecules, block oxidants from destroying tissue, and also help the body replace important antioxidants, like glutathione, which is a master antioxidant in our body. Lingonberry has also been shown to increase red blood cell and liver enzymes needed for antioxidant protection. We need antioxidants to protect vessels and nerve tissue, and also to help decrease the damage from inflammation. Proanthocyanidin extracts of lingonberries were also found to be effective against the bacteria Staphylococcus aureus, which can cause a wide variety of infections. (Mane *et al*. 2011). Alcohol, the most commonly consumed xenobiotic, generated reactive oxigen species (ROS).

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Liver is the primary organ responsible for the oxidation of ingested alcohol, but kidney may contribute to alcohol metabolism as well. (Lieber, 1994). Also, it is well known that alcohol can interfere with kidney function directly, through acute or chronic consumption, or indirectly, as a consequence of liver disease, so called hepatorenal syndrome.

The biochemical changes induced by alcohol consumption in the kidney are not well understood, though some clinical and experimental studies have been focused on the effects of alcohol feeding on renal function including gross and microscopic morphology (Broszka *et al.*, 2013).

The restauration of liver and kidney affected by ethyl alcohol, the most common and oldest known drug, under the influence of hepatic and renal-protectors of a vegetal extracts (*Vaccinium vitis idaea* extract) do not benefit of many data. Thus, this constituted an argument for us to carry out this study.

The aim of our study was to test the biological and protector potential effects of a 1:1 hidroalcoholic extract of *Vaccinium vitis idaea* L. on some biochemical blood parameters (serum urea and creainine) as well as kidney morphology affected by alcohol intoxication in white Wistar rats.

MATERIALS AND METHODS:

The experiment was made on mature white Wistar female rats, weighing 150 ± 10 g. Animals were divided into 4 experimental groups of 6 animals each, as follows: - Control group, C;

- E group, treated for 15 days with 1:1 *Vaccinium vitis idaea* L hidroalcoholic extract, 200 mg dry substance /100 g bw, by intragastric gavage, *á jeun.*;

- A group, intoxicated for 15 days with alcohol 50^0 in amount of 6 g/kg bw, by intragastric gavage, *á jeun.*

- AE group, treated for 15 days with 1:1 *Vaccinium vitis idaea* L hidroalcoholic extract, 200 mg dry substance /100 g bw and alcohol 50^0 (6 g/kg bw), by intragastric gavage, *á jeun.*

Table 1.
Urea and serum creatinine levels in the four experimental groups (C, E, A, and AE)

	C	E	A	AE
Urea (mg/dl)				
X±ESN P D%	51,35 ± 2,357 - -	51,72 ± 2,497 >0,5 +0,72%	40,61 ± 0,596 **<0,01** **-20,91%**	40,57 ± 1,157 **<0,01** **-20,99%**
Creatinine (mg/dl)				
X±ESN P D%	0,39 ± 0,0647 - -	0,37 ± 0,0567 >0,5 -5,12%	0,54 ± 0,067 **<0,05** **+38,46%**	0,47 ± 0,0567 **<0,05** **+20,51%**

Animals were obtained from the biobasis of „Iuliu Hatieganu" Medicine and Pharmacy University, Cluj-Napoca and kept under standardized zoohigienical conditions: in accordance to the European Communities Council Directive 2010/63/UE Directive of European Parlament and according to the approval of the Ethics Committee and Animal Protection for Experiments from the Institute of Biological Research, NIRDBS branch, Cluj-Napoca, Romania, (Decision 1/28.02.2013).

The *Vaccinium vitis idaea* hidroalcoholic extract (obtained by UMF Cluj) is a 1:1 hidroalcoholic extract (45^o), with a benzoic acid content of 40 mg/ 100g extract.

In the 16^{th} day, animals were killed by decapitation after a pre-anesthesia with ether.

Fragments of kidney were removed and fixed in Bouin liquid fixative and prepared for histology. Fragments were stained withhaematoxilin-eosin method for histological structure of kidney (Mureşan *et al.*, 1976).

The blood was colected and than processed according to analysed morphological or functional parameters.

We measured creatinine and serum ureea as indicators of renal function parameters. Thus, blood samples were immediately centrifuged, serum harvested and then frozen in Eppendorf vials. Measurements were made with biochemistry semiautomatic analyzer screen point type, with reagents - STATE - FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd Ramsey Minesota, USA 55303.

The biochemical data were statistically processed by means of Student's „t" test. Aberrant values were eliminated by means of Chauvenet's criterion. A probability value of $p \leq 0.05$ was considered.

RESULTS AND DISCUSSIONS:

The values of urea and serum creatinine, does not have any significant changes in the lingonberry extract treated group. In contrast, in both A and AE groups, the values of those two parameters change significantly, in opposite of each other, respectively: in A group urea level decreases with 20,91 % ($p< 0.01$) and serum creatinine increases with 38.46 % ($p< 0.05$), and in AE group urea decreases with 20,99 % ($p< 0.01$) and creatinine increases with 20,51 % ($p< 0.05$), the values being compared to control group. (Table 1).

It is well known that the concentration of creatinine and urea is an indicator of nephrotoxicity. The low clearance of creatinine and/or urea indicates a decrease in the ability of filtering and elimination of blood excretion products in urine at the kidneys level. While creatinine clearance decreases, the level in the blood increases and high creatinine level is an element in diagnosis of renal insufficiency (Saka *et al.*, 2012).

Treatment with lingonberry extract in E group led to a moderate decrease in creatinine concentration, associated with very slight decreases in the serum urea level, while subacute treatment with ethanol (Group A) within our experiment has resulted in an emphasized increase, statistically significant of serum creatinine concentration, indicating an installation process of the renal parenchyma lesions.

The vegetable extract administration with ethyl alcohol in AE group has determined a lower level of creatinine concentration, showing a protective effect of

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bioactive compounds contained in the plant extract. The protective effect is given by the ability of the bioactive compounds contained in fruits of lingonberry to normalise the renal function and to prevent the development of healthy kidney-related diseases.

While the renal function is protected, or at least the rate of decline is slowing down, the level of creatinine will be decreased (Oh, 2011).

Our results are in agreement with those of Nagami *et al*. (2013), who noticed that the administration of an ajurvedic-Vimliv compound, which is a complex containing a variety of medicinal plants rich in polyphenols, antrakinonic compounds, etc., had the effect of restoring serum urea and creatinine level increased as a result of intoxication with ethyl alcohol.

Serum urea values are dependent on the degree of protein metabolism, diuresis - urinary excretion-and of renal functional capacity. Low values of this parameter are found in children and pregnant women, in patients with serious liver disease which are hyperperfused, in cashexy or in protein malnutrition.

In E group serum urea value is almost identical to that of the control group, showing that Lingonberry extract does not exert adverse effects on kidney function. The decrease in serum urea level in A and AE groups might be due, on the one hand, to an imbalance of liver injury protein metabolism, affected by treatment with alcohol and alcohol related treatment + Lingonberry extract, and on the other hand, due to the presence of residues of flavonoids fruit lingonberries, which it has been demonstrated that can decrease serum uric acid concentration, protecting the kidney against hiperuricemia reduced by the adenine, (Su, 2012).

Histological study of kidney

In **C group** histological sections of kidney highlight the normal aspect of its morphology, with two distinct zones, an outer cortex and an inner medula (Fig.1). At the level of the renal cortex we can observe the presence of Malpighi corpuscles. The Malpighi corpuscle consists of glomerulus and Bowman's capsule. The first part of the tubular system is the proximal tubules, which is a continuation of Bowman's capsule and initially pursues a convoluted course (the proximal convoluted tubule). The straight portion of urinary tubules, loop of Henle, as well as interlacing segments of the distal convoluted tubule, ducts of Bellini and papillary ducts forms medullary substances of the kidney.

The renal Malpighi corpuscle is made of a cup-shaped epithelial component – the Bowman capsule, the renal glomerulus being formed from a bundle of capillaries with filtering role.

Bowman capsule has double walls with two rolling papers, parietal and visceral one, which goes on with one another at the vascular pole (place of entry and exit of the arterioles). The epitelial cells of Bowman's capsule are flat and simple, whose nuclei protruding slightly in capsular space. In contrast, the epithelial cells coating the glomerular capillary tuft are larger and have a highly specialized and unusual structure, named podocytes. The epithelial-lined space between the visceral shell and parietal shell of Bowman's capsule is called the capsular space (of filtering). The proximal convoluted tubules and the most part of the renal cortex, are delimited by a cubic epithelium, with large cells, having spherical nuclei located in the centre. The cells have apical "brush border" and mark the limits of a narrow lumen, the cellular limits being unclear because of the numerous lateral interdigitation.

Epithelial cells presents an intense acidophilic cytoplasm due to mitochondria giving an subnuclear striated appearance. The distal convoluted tubules are delimited by a cubic epithelium, having the lumen larger than the proximal convoluted tubules.

Epithelial cells are shorter and more numerous and intercellular limits are obvious. These cells do not show "brush border", cytoplasm being less acidophil. The conjunctivo-vascular tissue of cortical stroma is poorly represented. (Stevens & Lowe, 1993).

The animals subcronic treatment with ethylic alcohol (**A group**) determined the marked dilation of the lumen of the uriniferous tubules. The epithelium of the unriniferous tubules show aspects of vacuolar tubular dystrophy, granulo-vacuolar degeneration, the most affected components of the nephron are those located in the cortical. Thus the proximal tube and its distal segment are the most affected segments, the lesions were present in a variable number of nephrons. Some uriniferous tubes have epithelial desquamation, without affecting the integrity of the tubular basal membrane, which further promotes the process of regeneration and recovery.

At the glomerulus level have been found lesions characterized by a slight thickening of glomerular capillary loops associated with dilatation of interglomerulare spaces. Alcohol, which has vasodilator effect, determined the congestion of the lumen of the blood vessels, (Fig. 2).

In **E group**, treated with plant extract of lingonberry, there have not been reported morpho-structural changes of kidney, the general histological appearance being similar to C group (Fig. 3). Thus, the extract has not toxic effects on the glomerulus. Alongside, the renal tubular system shows a normal texture hence, the plant extract does not induce pathological changes in the proximal and distal tubules.

In **AE group**, as a result of lingonberries plant extract administration associated with ethyl alcohol, there was found an attenuation of alcohol-induced tubular dystrophic aspects. Tubular portions, including vacualiztion and desquamation phenomena of urinary tubule epithelium, may still persist in some areas of cortex. But general appearance of kidney structure suggests a protective effect of lingonberry plant extract in maintaining of the kidney structural integrity (Fig. 4).

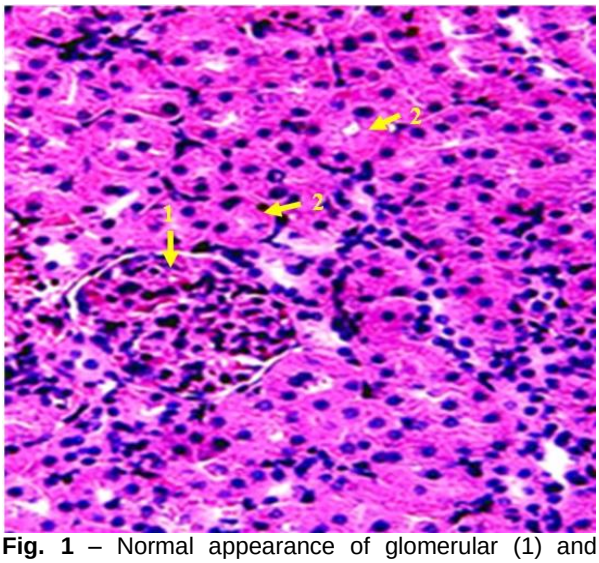

Fig. 1 – Normal appearance of glomerular (1) and uriniferous tubules (2) structures in C group, (x 400)

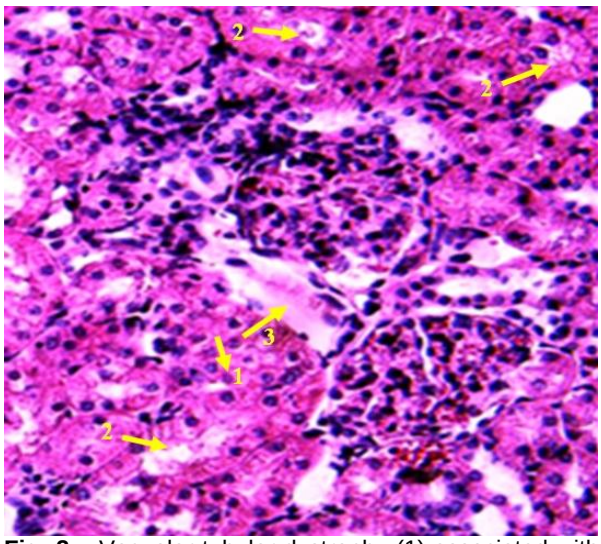

Fig. 2 – Vacuolar tubular dystrophy (1) associated with emphasized epithelial uriniferous tubules desquamation (2),dilatation of inter-glomerular spaces in A group (3), (x 400)

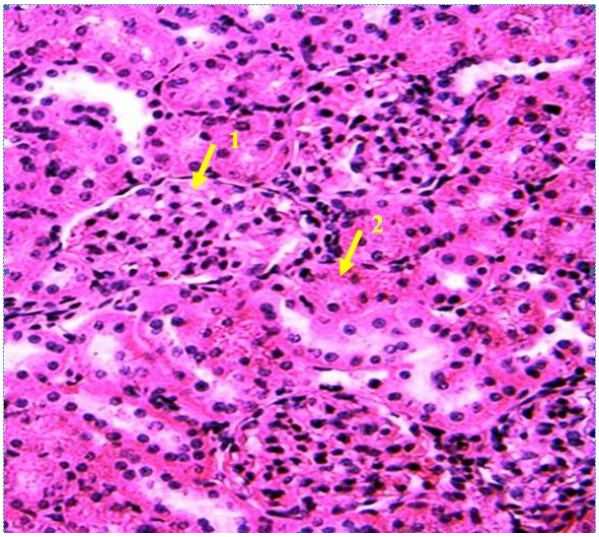

Fig. 3 – Normal appearance of glomerular (1) and uriniferous tubules (2) structures in E group, (x 400)

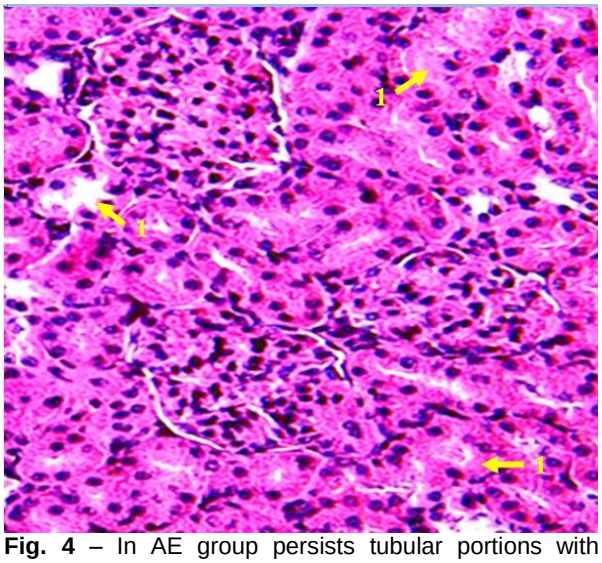

Fig. 4 – In AE group persists tubular portions with epithelial esquamation (1), (x 400)

Subacute intoxication with ethyl alcohol in A group, induced lesions of medium intensity in the kidney, being represented by aspects of clear and vacuolare dystrophy of urinary tubules, associated with emphasized dilatation of the urinary lumen tubules. Part of the the proximal and distal convoluted tubules exhibit epithelial cells increased in volume, epithelial desquamation, as well as congestion of the blood vessels. Morphopathological changes are more accentuated in the renal cortical.

The reported aspects have reversible character, because the basal tubular membrane retains its structural integrity, thus favouring the process of regeneration and recovery. At the glomerulus level, injuries were also reduced, being characterized by a thickening of the glomerular capillary loops, associated with dilatation of interglomerular spaces.

In E group, morpho-structural changes of kidney have not been signalled, demonstrating the fact that vegetable extract of Vaccinium vitis idaea is free of toxicity and does not induce secondary undesirable side effects at kidney level.

In AE group, as a result of lingonberry plant extract administration associated with ethyl alcohol, there have been found an attenuation of alcohol-induced tubular dystrophic aspects, being expressed by a moderate recovery of kidney structure. In some cortical areas may persist, however, tubular portions including vacuolation and desquamation phenomena of urinary tubule epithelium, but the general appearance of the kidney structure suggests a protective effect of the lingonberry plant extract in maintaining structural integrity of kidney.

Our results have demonstrated that administration of bioactive vegetable extract of *Vaccinium vitis idea* has determined repairing effects at the level of the renal tubular epithelial cell membranes in the alcohol intoxicated rats, suggesting a protective potential, respectively, and the stimulation the kidney structural regeneration in the alcoholic intoxication conditions.

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CONCLUSIONS:

Excessive alcohol consumption can have profound negative effects on the kidneys and their function, respectively on the dynamics of the creatinine and serum urea in both alcohol treated group and alcohol + lingonberry treated group. In the AE group the alcohol effects were more diminished, which means that our extract has protective effects against the alcohol iduced injuries in kidney.

Concerning morphological modification, the results have shown that administration of bioactive vegetable extract of*Vaccinium vitis idea* has determined repairing effects at the level of the renal tubular epithelial cell membranes in the alcohol intoxicated rats. This suggests a protective potential, and, respectively, the stimulation of the kidney structural regeneration in the alcoholic intoxication conditions

Single administration of vegetable extract of lingonberry not caused toxic effects on the kidney function, while treating animals with lingonberry extract had beneficial effects in preventing and protecting parameters of kidney function taken into study, in terms of alcoholic intoxication.

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