The Use of Sea Buckthorn (*Hippophae rhamnoides* L.) and Milk Thistle (*Silybum marianum* L.) in Alloxan Induced Diabetes Mellitus in Rats

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**Abstract**

The study emphasized the effect of *H. rhamnoides* and *S. marianum* 6% aqueous extract on blood sugar level in Alloxan induced diabetes mellitus in rats. The rats (200g and 3 months age) were divided in five groups: one non-diabetic control and four experimental groups with induced diabetes mellitus after 40 mg/kg b.w. intravenous administration of 2% Alloxan. One group was kept as diabetic control and in the other three groups was administered 6% aqueous extracts of *H. rhamnoides*, *S. marianum* or a combination of the extracts during seven weeks. The stronger effect was obtained in case of *H. rhamnoides* (Sea buckthorn) extract administration followed by the *S. marianum* (Milk thistle) extract. The combination of the two extracts proven to have a stronger effect than the extracts given separately.

**Keywords**: Alloxan, seabuckthorn, diabetes, milkthistle, rats.

1. Introduction

One of the most concerning health problems all around the world was considered diabetes mellitus [1], a metabolic disorder in the endocrine system in which patients suffering from it are not able to produce or properly use insulin in the body, so they have a high content of blood glucose [2]. In the last few decades, were used numerous animal models to study diabetes mellitus and, also to test different anti-diabetic agents [3, 4].

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetraone) is a well-known diabetogenic agent which is capable to induce experimental Type I diabetes in animals [3, 5].

Sea buckthorn (*Hippophae rhamnoides* L.), part of Elaegnacea family is a thorny deciduous plant which can be found in several countries of Europe and Asia. All parts of this plant contain a large number of bioactive substances with high medicinal and nutritional properties. Sea buckthorn seed oils, berries, leaves and bark are well known for their medicinal properties due to the high contents of antioxidative substances present in this plant [6, 7]. Sea buckthorn berries are the rich source of vitamins A, C, E, and K, flavonoids, carotenoids, organic acids and oils [8].

*Silybum marianum*, an annual or biennial herb that belongs to the *Asteraceae* family, in which the main active ingredients are alkaloids, flavonoids, saponins, tannin, and several flavonolignans collectively known as silymarin [9]. The silymarin, being an very good antioxidant, has been proven to reduce blood cholesterol, to promote liver cell regeneration and to prevent cancer [10].

The aim of the present study was to evaluate the beneficial effects of Sea buckthorn and milk
thistle, especially the combination between this two extracts, in rat’s diabetes mellitus.

2. Materials and methods

Animals
Wistar albino rats (n = 25), 3 months age and weighting 200 g, were purchased from the Animal House of University of Medicine and Pharmacy “Victor Babeș” Timisoara, Romania. The rats, housed in plastic cages, were kept for one week as acclimatization period before the start of experiment, at constant room temperature of 25±2°C, 12 h light/dark cycle and fed ad libitum with standard diet. They were handled in accordance with the standard guide for the care and use of laboratory animals.

Plant material
The plant material was purchased from natural plants shop. Classic extraction was performed from the Sea buckthorn berries and Milk thistle seeds mixing approx. 0.4 mm particle size with distillated water in weight/volume ratio of 0.6/10 (w/v). The mixture was heated at 90°C for 10 minutes and after filtered [11].

Experimental model
The rats from experimental groups were injected i.v. (in tail vein) with Alloxan (Sigma-Aldich, St. Louis, USA) 2% in dose of 40 mg/kg bw., according to the protocol of Carvalho et al. [5]. Seven days after Alloxan administration the glycaemia was analyzed using a portable glucometer ACCU-CHEK Active, model GC (ROCHE, Mannheim, Germany) with specific stripes. The rats that present a glycaemia over the 135 mg/dl were considered diabetics and those who overpass the 200 mg/dl were considered to have severe diabetes. The considered diabetic rats were randomly divided in four groups (n=5) as follows: DC – diabetic control group receiving distilled water, HR – group receiving H. rhamnoides 6% aqueous extract, SM – group receiving S. marianum 6% aqueous extract, HR+SM – receiving combination of 6% extracts. The fifth group (n=5), non-diabetic control (NC), receiving also as DC only distilled water. The body weight and blood sugar level was measured twice a week during seven weeks.

3. Results and discussion

Alloxan has been used to induce experimental diabetes due to the it’s selective destruction of the insulin-producing pancreatic beta-islets, having the capacity to produce a multiphase blood glucose response when is injected into an experimental animal, being accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultra structural beta cell changes and, finally leading to necrotic cell death [12].

In the present study, we observed a not significant increase (p> 0.05) of body weight in diabetic compared to non- diabetic control (Figure 1). In groups exposed to plants extracts the body weight decreased compared to the both control groups in H. rhamnoides exposure starting with the second week after exposure and in S. marianum exposure starting with third week after administration (p>0.05). The decrease of body weight was observed also in the group that received the combination of plant extract compared to the both control groups (p<0.05).

Regarding the blood sugar levels in present study we observed in diabetic control a glycaemia remaining to an approx constant mean level during the seven weeks that overpass 150 mg/dl , excepting second week when the glycaemia dropped to 135 mg/dl (Figure 2). When the extract of H. rhamnoides 6% was administered, the blood sugar levels were decreased starting with the third week of administration, recording significant differences (p>0.05) until the end of the experiment.

The S. marianum 6% extract showed a good antiglycaemic effect reducing the blood sugar levels in rats exposed compared to diabetic control. This decrease was significant starting with the fifth week of administration (p<0.05), recording a tendency to reach the values recorded in non-diabetic control group.

The clinical effect of Sea buckthorn was more evident compared to the Milk thistle effect, but the differences between these groups were statistically not significant (p>0.05).
Being known the other mechanism of Alloxan action and, possible mechanism of diabetes induction, by generation of reactive oxygen species (ROS) and superoxide radicals [12, 13],

![Figure 1. Body weight dynamics in rats exposed to H. rhamnoides and S. marianum 6% extracts](image1)

![Figure 2. Blood sugar dynamics in rats exposed to H. rhamnoides and S. marianum 6% extracts](image2)

the possible explanation of both extracts effects could be the polyphenols content which counteract the ROS [6, 14, 15, 16].

Wang et al. [14] in a review regarding the *H. rhamnoides* effect on diabetes pointed out that oxidative stress plays an important role in the etiology of diabetes and its complications, and for this new methods of treatment for prevention and control of this disease are focused on stopping the oxidative stress.

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In the group that received mixture of plants extract, the blood sugar level decreased significantly after the third and fourth week of administration and was maintained until the end of the experiment (p<0.01). Abu-zaiton [17] noted that the administration of *S. marianum* extract at dose 400 mg/kg to diabetic rats was able to decrease significantly the glucose levels. In other studies the authors conclude that *S. marianum* seed extract administration to diabetic cirrhotic patients resulted in the reduction of insulin resistance and the need for exogenous insulin administration [18].

Zhang et al. [15, 16] noted that administration of 400 mg/kg aqueous extract of seabuckthorn seed residues in rats with diabetes was followed by a hypoglycemic, hypotriglyceridemic and antioxidant effects.

### 4. Conclusions

The present study pointed out the reduction of glycaemia by administration of *H. rhamnoides* 6% and *S. marianum* 6% extracts. The better results were obtained in case of *H. rhamnoides* 6% extract. The combination of the two extracts proven to have a stronger effect than the extracts given separately, thus we recommend this as a possibility of homeopathic control of diabetes.

### References

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