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

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Central and South Eastern Europe

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- **Diabetes mellitus** (more commonly referred to as "diabetes,") is a chronic disease associated with abnormally high levels of the sugar glucose in the blood.
- Diabetes is due to one of two mechanisms:
 - a. Inadequate production of insulin (which is made by the pancreas and lowers blood glucose), or
 - b. Inadequate sensitivity of cells to the action of insulin.

Diabetes is estimated to affect aprox. 380 million people and it is estimated that in 2030, over 500 million will be suffering from diabetes worldwide

Plants

Hippophae rhamnoides

Sea buckthorn berries are the rich source of vitamins A, C, E, K, flavonoids, carotenoids, organic acids and oils

Can be used for:

- treating arthritis, gastrointestinal ulcers, gout, and skin rashes caused by infectious diseases;
- improving blood pressure and lowering cholesterol;
- preventing and controlling blood vessel diseases;
- boosting immunity.

preventing infections, improving sight, and slowing the aging process.

- treating asthma, heart disorders and high cholesterol;
- reducing illness due to cancer, as well as limiting the toxicity of chemotherapy;



Plants

Silybum marianum

the main active ingredients are: alkaloids, flavonoids, saponins, tannin, and several flavonolignans collectively known as silymarin.

The silymarin, being an very good antioxidant, has been proven to:

- reduce blood cholesterol,
 - promote liver cell regeneration
 - prevent cancer



Experimental prothocol

The animals

Wistar albino rats (n = 25), 3 months age and weighting 200 g,

- 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
- 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

- purchased from natural plants shop
- classic extraction: 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







Experimental model

- the rats were injected i.v. (in tail vein) with Alloxan 2% in dose of 40 mg/kg bw. - after 7 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 over 200 mg/dl were considered to have severe diabetes.





the considered diabetic rats were randomly divided in four groups (n=5):

- **DC** diabetic control group receiving distillated 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 distillated water.

The body weight and blood sugar level was measured twice a week during seven weeks.



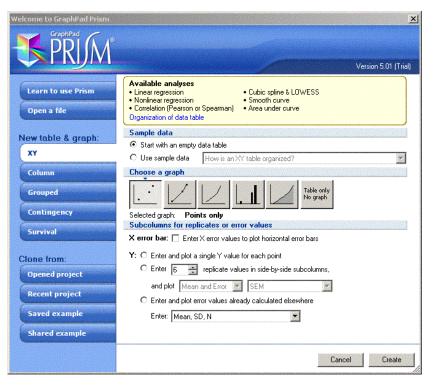


Statistical analysis

GraphPad Prism 5.0 for Windows (San Diego, USA).

The measured parameters were expressed as mean \pm SEM.

For evaluation of differences between studied groups, two-way ANOVA (Bonferroni's correction), considering statistical difference when p<0.05.



Results

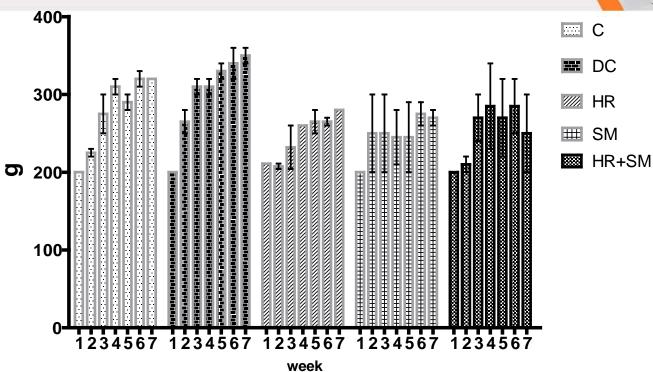


Figure 1. Body weight dynamics in rats exposed to *H. rhamnoides* and *S. marianum* 6% extracts



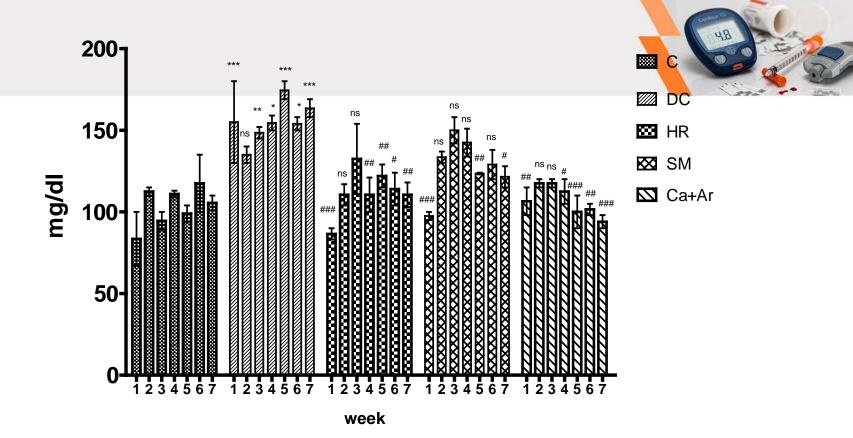
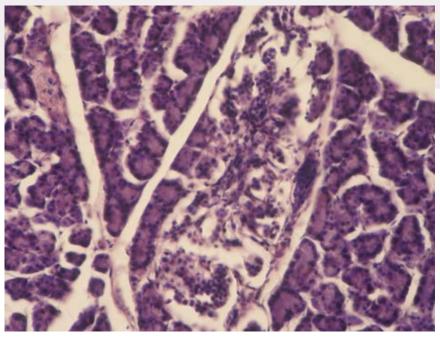


Figure 2. Blood sugar dynamics in rats exposed to *H. rhamnoides* and *S. marianum* 6% extracts

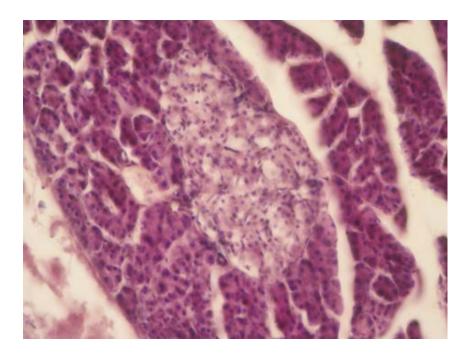
Comparative to C group: ns - not significant, * - p<0.05, ** - p<0.01, *** - p<0.001 Comparative to DC group: ns - not significant, # - p<0.05, ## - p<0.01, ### - p<0.001



Pancreas section in treated rats, stained H&E, ob. 40 – improvement of tissue architecture

Pancreas section in diabetes induced rats stained H&E, ob. 40 –

- leukocyte infiltrate,
- edema



Conclusions



- 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.



THANK YOU for attention!

