

The Use of Yellow Dock (*Rumex crispus* L.) and Goji Berry (*Lycium barbarum* L.) in Alloxan Induced Diabetes Mellitus in Rats

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Abstract

The present study emphasize the effect of *R. crispus* and *L. barbarum* 6% aqueous extract on blood sugar level in Alloxan induced diabetes in rats. The rats 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 *R. crispus*, *L. barbarum* or a combination of the extracts during seven weeks. The better results were obtained in case of *L. barbarum* (goji) extract administration followed by the *R. crispus* (yellow dock) extract. The combination of the two extracts has proven to have a weaker effect than the extracts given separately.

Keywords: Alloxan, dock, diabetes, goji, rats.

1. Introduction

In biology, in the last few decades, were used numerous animal models to study diabetes mellitus and, also to test different anti-diabetic agents [1, 2].

One of the most potent method to induce experimental diabetes mellitus is chemical induction by Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) which is an well-known diabetogenic agent capable to induce experimental Type I diabetes in animals [1, 3].

Rumex crispus L. (yellow dock) is an herbaceous perennial plant and is widespread over humid or mesophyllous open areas with grass or ruderal vegetation, mostly in acid (containing silicate) soils. Yellow dock (*R. crispus*) considered as an invasive weed in many areas, it spreads through

the fruits contaminating crop seeds, and sticking to clothing of fleece [4].

The activity of *R. crispus* is due to the various groups of chemical constituents such as: flavonoids, tanning agents (aprox 5% in leaves and 20% in fruits), phenolic acids, anthraquinones, polyunsaturated fatty acids and other substances as is oxalic acid [5-7].

Lycium barbarum L. (goji) is a deciduous shrub one to three meters high with lanceolate to ovate leaves. The fruits are oblong, orange to dark red berries measure up to 2 cm and possess a bitter to sweet taste [8].

The Goji fruits contain numerous chemical components including betaine, phenols, β -carotenes and polysaccharides [9].

The goal of the present study was to evaluate the beneficial effects of dock and goji in rat's diabetes mellitus.

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2. Materials and methods

Animals

Young adult Wistar albino rats (n = 25) weighting 200 g, were purchased from the Animal House of University of Medicine and Pharmacy “Victor Babeş” 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 $25\pm 2^{\circ}\text{C}$, 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 leaves of *Rumex crispus* was collected from the field of Timis County, Romania and was compared for identification with a herbarium specimen deposited in the Department of Vegetal Biology and Medicinal Plants, Faculty of Veterinary Medicine Timisoara, Romania. The goji berry was purchased from natural plants shop. Classic extraction was performed from the rumex leaves and goji berry mixing approx. 0.4 mm particle size with distilled water in weight/volume ratio of 0.6/10 (w/v), in Erlenmeyer flasks. The mixtures were heated at 90°C for ten minutes and after filtered [10].

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 described by Carvalho et al. [4]. 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, RC – group receiving *R. crispus* 6% aqueous extract, GB – group receiving Goji berry 6% aqueous extract, RC+GB – receiving combination of 6% extracts. The fifth group (n=5) was 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.

Statistical analysis

The measured parameters were expressed as mean \pm SEM. For the evaluation of differences between studied groups, one-way ANOVA with Bonferroni's correction was used, considering statistical difference when $p < 0.05$ or lower. The software was GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, USA).

3. Results and discussion

One of the most concerning health problems all around the world was considered diabetes mellitus [11]. Due to the selective destruction of the insulin-producing pancreatic beta-islets Alloxan has been used to induce experimental diabetes, producing a multiphase blood glucose response when is injected into to 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 control compared to non-diabetic control (Figure 1). In case of studied plant administration the body weight decreased compared to the both control groups in *R. crispus* exposure starting with the fifth week after exposure and in *L. barbarum* exposure starting with fourth week after administration ($p < 0.05$, $p < 0.01$). The same dynamics was observed 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 a approx constant mean level during the seven weeks that overpass 150 mg/dl (Figure 2). When the extract containing *R. crispus* 6% was administered the blood sugar levels were decreased especially in the third and fourth week of administration but the differences were not significant ($p > 0.05$), remaining still not statistically different from non diabetic control group ($p > 0.05$).

The goji berry 6% extract showed a good antiglycaemic effect reducing the blood sugar

levels in rats exposed to it compared to diabetic control. This decrease was significant starting with the third week of administration ($p < 0.05$), recording a tendency to reach the values recorded in non-diabetic control group. Even if clinically the effect of *L. barbarum* extract was evidently more efficient than the extract of *R.*

crispus the differences were statistically not significant ($p > 0.05$).

As a possible explanation of both extracts effects could be the polyphenols content, known being the other mechanism of Alloxan action and, possible mechanism of diabetes induction, by

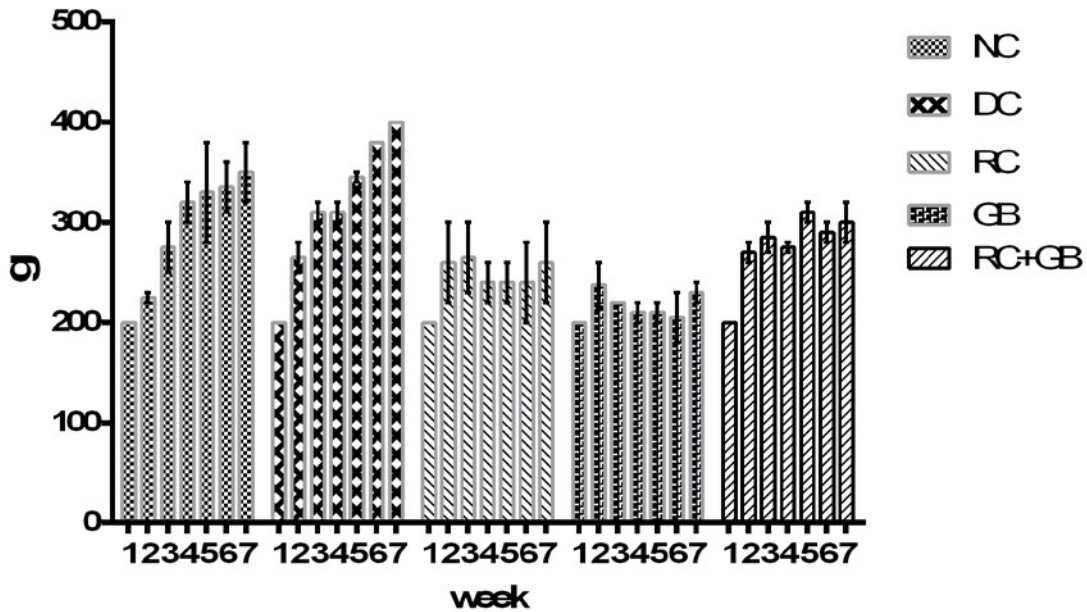


Figure 1. Body weight dynamics in rats exposed to *R. crispus* and *L. barbarum* 6% extracts

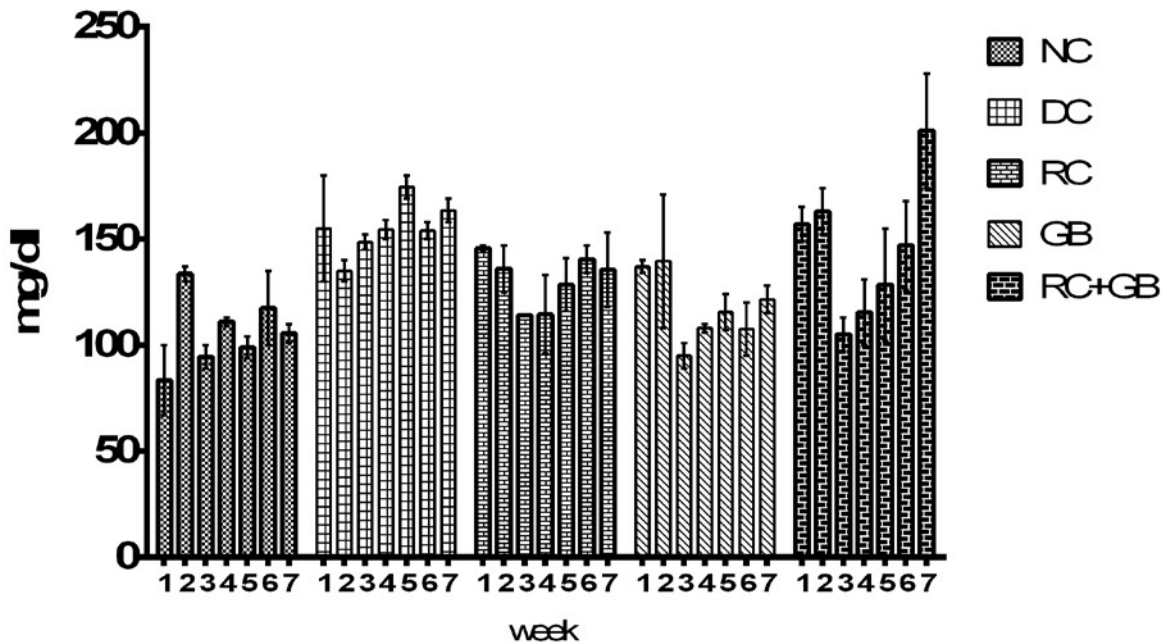


Figure 2. Blood sugar dynamics in rats exposed to *R. crispus* and *L. barbarum* 6% extracts

generation of reactive oxygen species (ROS) and superoxide radicals [12, 13].

In the group that received mixture of plants extract, the blood sugar level was decreased only in the third and fourth week of administration, the differences being not significant ($p > 0.05$). Starting with the fifth week the blood sugar level started to increase, the possible explanation of this situation could be the tannin content of both plants that limit the absorption or the taste modification of the infusion having as consequences the consumption limitation. Ahmed et al. [14] noted that extracts of *Vinca rosea* at high dose (500 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (300 mg/kg) in alloxan-induced diabetic rats. Kumar et al. [15] showed that administration of *Rumex maritimus* in doses of 500 mg/kg in diabetic rats

during 21 days reduced the blood sugar level, the results being similar to ours for the first for weeks of administration.

4. Conclusions

The present study pointed out the reduction of glycaemia by administration of *R. crispus* 6% and *L. barbatum* 6% extracts. The better results were obtained in case of *L. barbatum* (goji) 6% extract. The combination of the two extracts has proven to have a weaker effect than the extracts given separately.

References

1. Etuk, E.U., Animals models for studying diabetes mellitus, Agric Biol J N Am, 2010, 1, 130 – 134
2. Srinivasan, K., Ramarao, P., Animal models in type 2 diabetes research: an overview, Ind J Med Res, 2007, 125, 451-72
3. Carvalho, E.N., Carvalho, N.A.S., Ferreira, L.M., Experimental model of induction of *diabetes mellitus* in rats, Acta Cir Bras [serial online] Vol.1 8 Special Edition, 2003. Home page address: <http://www.scielo.br/acb>
4. Maksimović, Z., Kovačević, N., Lakušić, B., Čebović, T., Antioxidant activity of yellow dock (*Rumex crispus* L., Polygonaceae) fruit extract, Phytother. Res., 2011, 25, 101–105
5. Jimoh, F.O., Adedapo, A.A., Aliero, A.A., Afolayan, A.J., Polyphenolic contents and biological activities of *Rumex ecklonianus*, Pharmaceutical Biology, 2008, 46, 333–340
6. Smolarz, H.D., Wegiera, M., Matyjasik, J., Fatty acids composition in fruits of *Rumex* L. genus. Annales UMCS Sectio DDD, 2008, 21, 133–137
7. Wegiera, M., Grabarczyk, P., Baraniak, B., Smolarz, H., Antiradical properties of extracts from roots, leaves and fruits of six *Rumex* L. species, Acta Biologica Cracoviensia Series Botanica, 2011, 53, 1, 125–131
8. Poterat, O., Goji (*Lycium barbarum* and *L. chinense*): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity, Planta Med., 2010, 76, 7–19
9. Song, Y., Xu, B., Diffusion profiles of health beneficial components from Goji berry (*Lyceum barbarum*) marinated in alcohol and their antioxidant capacities as affected by alcohol concentration and steeping time, Foods, 2013, 2, 32-42
10. Alupului, A., Calinescu, I., Lavric, V., Ultrasonic vs. microwave extraction intensification of active principles from medicinal plants, AIDIC Conference Series, 2009, 09, 1-8, doi:10.3303/ACOS0909001
11. Kruger, D.F., Lorenzi, G.M., Dokken, B.B., Sadler, C.E., Mann, K., Valentine, V., Managing diabetes with integrated teams: maximizing your efforts with limited time, Postgrad Med, 2012, 124, 64-76
12. Ankur, R., Shahjad, A., Alloxan Induced Diabetes: Mechanisms and Effects, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012, 3, 2, 819-823
13. Das, J., Vasan, V., Sil, P.C., Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis, Toxicol Appl Pharmacol, 2012, 258, 296-308
14. Ahmed, M.F., Kazim, S.M., Ghori, S.S., Mehjabeen, S.S., Ahmed, S.R., Ali, S.M., Ibrahim, M., Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats, International Journal of Endocrinology, Volume 2010, doi:10.1155/2010/841090
15. Kumar, S.D., Surajit, K.G., Sorra, S., Joyeeta, T., Dipankar, S., Assessment of In vitro Antioxidant potential and In vivo Anti-diabetic Activity on Streptozotocin-induced Diabetic Rats of *Rumex maritimus*L., Asian Journal of Biochemical and Pharmaceutical Research, 2014, 4, 2, 164-170.