ANTIDIABETIC EFFECT OF DIATARIN CAPSULES ON ALLOXAN-INDUCED TYPE 2 DIABETES IN MICE

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Diabetes mellitus has become a major public health threat across the globe. Currently, antidiabetic therapies are based on synthetic drugs which have side effects; as such, there has been a great amount of research done to determine the role of natural products in the treatment of diabetes. Diatarin capsules is a product of Ipharm Vietnam Pharmaceutical Joint Stock Company with the components from herbal ingredients. This study aimed to investigate the hypoglycemic activity of Diatarin capsules on Alloxan (ALX) induced diabetic mice. Before mice were injected intraperitoneally ALX at 180 mg/kg, they were fed a high fat diet for 8 weeks. Mice were administered orally Diatarin capsules at 326.4 mg/kg b.w/day for 2 weeks. The results revealed that Diatarin capsules at 326.4 mg/kg b.w/day ameliorated the effects of ALX on the glucose, LDL-C, total cholesterol levels and histopathological morphology of the liver. This suggested that Diatarin capsules could be a potential agent for treating diabetes mellitus.

Keywords: Diatarin capsules, hypoglycemic activity, high fat diet, alloxan.

I. INTRODUCTION

According to the IDF Diabetes Atlas, since the first edition in 2000, the estimated prevalence of diabetes (type 1 and type 2 combined, both diagnosed and undiagnosed) in people aged 20 – 79 years has risen from 151 million (4.6% of the global population then) to 463 million (9.3%) today. It is predicted that 578 million people (10.2% of the population) will have diabetes by 2030. That number will jump to a staggering 700 million (10.9%) by 2045. Globally, diabetes is among the top 10 causes of death. The health consequences of diabetes will increasingly overwhelm the health care systems due to the severity of the long- term complications of diabetes. [1] Several synthetic drugs such as sulfonylureas and biguanides are available as an oral hypoglycemic agent to lower blood glucose level in diabetics. However, their administration might produce side effects to the patients. [2] Therefore, finding of new prevention strategies and treatments for diabetes is urgent. Nowadays, many researches begin to explore natural plant products with anti-diabetic potential as an alternative therapy. Since there are plenty of natural plant products that exhibit anti-diabetic potential, the research can focus on herbal remedy to support diabetes treatment. The plant kingdom offers a wide field to look for effective oral hypoglycemia.

Diatarin is a product manufactured by the third generation nanotechnology. Its main component is the targeted delivery system [GA (berberin - curcumin)] (containing berberin and

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curcumin attaching glycyrrhizic acid molecules to create liver cell – targeted delivery) with 50-70 nm diameters nanoparticles. Berberin was isolated from *Coscinium fenestratum* (Gaertn.) Colebr and curcumin was isolated from *Curcuma longa* L. Besides, Diatarin capsules also contained Lagerstroemia speciosa L. extract; rutin and quercetin isolated from *Styphnolobium japonicum* L. To date, many studies on the antidiabetic effect of each component have been reported. [3,4,5,6] However, there has been no report available of their effects in combined preparation as Diatarin capsules. Therefore, this study aimed to evaluate the hypoglycemic activity of Diatarin capsules in alloxan-induced type 2 diabetic mice.

II. SUBJECTS AND METHODS

1. Experimental medicine

Diatarin is manufactured by Ipharm Vietnam Pharmaceutical Joint Stock Company. It is a capsule formulation and each capsule contained 250 mg the targeted delivery system [GA (berberin – curcumin] with berberin isolated from Coscinium fenestratum (Gaertn.) Colebr and curcumin isolated from Curcuma longa L, 75 mg total flavonoid (75% rutin and 25% quercetin) isolated from *Styphnolobium japonicum* L. and 15 mg *Lagerstroemia speciosa* L.

In diabetes patients, the recommended dosage of Diatarin is 2 capsules each time, 2 - 3 times per day.

2. Experimental animals

Healthy Male *Swiss* albino mice (weighing 21 - 25 g and age of 8 - 12 weeks) were purchased from the National Institute of Hygiene and Epidemiology Vietnam (NIHE). The mice were fed with standard pellet diet (standard animal feed, NIHE) and water ad *libitum.* After randomly grouping and before initiation of the experiment, animals were acclimatized to the laboratory conditions for 7 days at the laboratory of Department of Pharmacology, Hanoi Medical University.

3. Machines and chemicals

- Alloxan 10 g (Sigma-Aldrich, Singapore)
- Diamicron 30 mg MR tablets (gliclazide) (Les Laboratoires Servier, France).
- Blood glucose monitoring system On Call EZII (ACON Biotech, USA)
- Animal blood counter Vet Exigo (Bonle Medical AB, Sweden)
- Chemistry analyzer Erba and Test strips: blood triglyceride, HDL-C, total cholesterol (DIALAB GmbH, Austria).

4. Researching method

The study was divided into two stages[7]:

**** The first stage:***

Before studying, fasting blood glucose level were measured.

+ Group I: Normal group (n = 10): Mice were fed normal fat diet (NFD)

+ Group II Diabetic group (n = 60): Mice were fed high fat diet (HFD) within 8 weeks following Fabiola and Srinivasan method with 43% saturated fat combined siro fructose 55%[8].

After 8 weeks, fasting blood glucose level was measured in both groups . Mice in group II were injected intraperitoneally ALX at 180 mg/kg. Mice in group 1 were injected intraperitoneally 0.9% NaCl. After 72 hours, blood glucose level (t_0) was checked in mice of group II; the mice were considered diabetic if their blood glucose level was greater than 8.0 mmol/L and subsequently, they were divided randomly into groups from group 2 to group 4 as below.

**** The second stage:*** The study was carried out in a continuous 14-day period. Mice were divided into 4 groups of 10 animals:

- Group 1 (control): NFD + distilled water.
- Group 2 (model): HFD + an intraperitoneal injection of ALX 180mg/kg + distilled water.

- Group 3 (gliclazide at 80 mg/kg/day): HFD + an intraperitoneal injection of ALX 180 mg/kg + oral administration of gliclazide at 80 mg/kg/day.

- Group 4 (Diatarin at 326.4 mg/kg/day): HFD + an intraperitoneal injection of ALX 180 mg/kg + oral administration of Diatarin at 326.4 mg/kg/day.

After 1 week (t1) and 2 weeks (t2) of treatment, the mice fasting blood glucose level was tested. At the end of experiment, blood lipid indexes (total cholesterol (TC), triglyceride (TG), HDL-Cholesterol, LDL-Cholesterol) were measured as well as subjected to a full gross necrospy. Liver and pancreas of 30% of the mice of each group were removed for histopathological examinations.

5. Statistical analysis

Data were analyzed by T-test in Microsoft Excel software version 2010. Data are presented as Mean ± Standard Deviation. A p-value less than 0.05 is statistically significant.

III. RESULTS

1. Effect of Diatarin capsules on the weight of mice

The result of figure 1 showed that after 4 weeks, 6 weeks and 8 weeks, the weight of HFD fed mice increased significantly compared with baseline ($p < 0.001$). As compared with NFD fed mice, at all time points, there was a subtantial increase in the weight of HFD fed mice ($p < 0.001$**).**

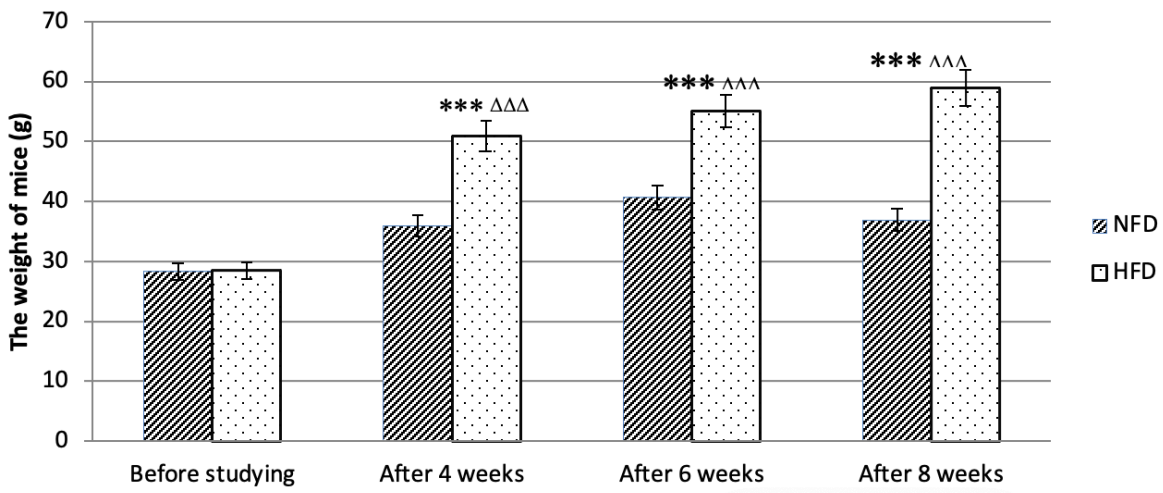

Figure 1. The chance in the weight of mice

NFD: normal fat diet, HFD: high fat diet

****: p < 0.001 compared with the weight before studying;*

ΔΔΔ: p < 0.001 compared with the weight of group fed NFD

2. Effect of Diatarin capsules on blood glucose level

As shown in Table 1, at 72 hours after ALX injection, the blood glucose level of HFD fed mice (group 2) increased significantly as compared with the baseline and before ALX injection ($p < 0.001$). Besides, at 8 weeks of HFD and 72 hours after ALX injection, the blood glucose level of mice in group 2 posed a significant increase as compared with group 1 ($p < 0.01$ and $p < 0.001$).

Table 1. The change in blood glucose level of mice

Time	Blood glucose level (mmol/l) ($\overline{X}$ ± SD) (n = 10)		p (compared with group 1)
	Group 1: NFD	Group 2: HFD	
Before studying	4.80 ± 0.88	5.25 ± 1.42	> 0.05
After 8 weeks	4.95 ± 0.85	7.13 ± 1.96	< 0.01
72 hours after ALX injection	5.35 ± 0.65	10.60 ± 2.76***+++	< 0.001

**** p < 0.001 compared with the time point "Before studying"*

+++ p < 0.001 compared with the time point "After 8 weeks".

Diatarin capsules at the dose of 326.4 mg/kg/day administered orally for 2 weeks reduced blood glucose level in comparison to the model group ($p < 0.01$ after 1 week and $p < 0.05$ after 2 weeks). No significant difference was observed in blood glucose level between mice treated with Diatarin capsules and gliclazide (Table 2).

Table 2. Effect of Diatarin on blood glucose level of mice after 2 weeks of treatment

Group	Blood glucose level (mmol/l) ($\overline{X}$ ± SD) (n = 10)		
	t_0 (before treatment)	t_1 (after 1 week of treatment)	t_2 (after 2 weeks of treatment)
Group 1 (control)	5.35 ± 0.65	5.96 ± 0.84	5.70 ± 0.76
Group 2 (model)	11.10 ± 2.21***	11.00 ± 2.98***	9.58 ± 2.24***
Group 3 (gliclazide 80 mg/kg/day)	10.67 ± 1.94***	8.40 ± 1.17Δ	7.59 ± 1.34Δ
Group 4 (Diatarin 326.4 mg/kg/day)	10.33 ± 2.54***	7.59 ± 2.07ΔΔ	7.04 ± 1.96Δ

**** p < 0.001 compared with control group;*

Δ,ΔΔ p < 0.05, p < 0.01 compared with model group

3. Effect of Diatarin capsules on blood lipid concentrations

Table 3 illustrated lipid disorders of group 2 (model) through the high concentration of TC, TG and LDL-C. In the group treated with Diatarin capsules, there was a significant decrease in TC and LDL-C concentration as compared with group 2 ($p < 0.01$). However, no significant difference was observed between the group treated Diatarin capsules and group 2 in terms of TG and HDL-C concentration ($p > 0.05$).

Table 3. Effect of Diatarin on blood lipid concentrations after 2 weeks of treatment

Group	Blood lipid concentration (mmol/L)			
	TC	TG	HDL-C	LDL-C
Group 1 (control)	2.44 ± 0.30	0.86 ± 0.18	0.87 ± 0.11	1.18 ± 0.21
Group 2 (model)	2.88 ± 0.42*	1.09 ± 0.13**	0.86 ± 0.12	1.52 ± 0.41*
Group 3 (gliclazide 80 mg/kg/day)	2.72 ± 0.15	0.95 ± 0.10$^{\Delta}$	0.91 ± 0.17	1.38 ± 0.24
Group 4 (Diatarin 326.4 mg/kg/day)	2.30 ± 0.25$^{\Delta\Delta}$	1.00 ± 0.20	0.83 ± 0.19	1.02 ± 0.25$^{\Delta\Delta}$

, p < 0.05, p< 0.01 compared with control group*

Δ,ΔΔ p < 0.01 compared with model group

TC: total cholesterol, TG: triglyceride, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol

4. Effect of Diatarin capsules on micro-histopathological study of liver and pancreas

As shown at figure 2 and figure 3, mice posed severe hepatic degeneration in group 2 (model). In the groups treated with gliclazide and Diatarin capsules, there was a significant improvement in liver histopathological examination compared with the model group. No significant difference was observed in histopathological study of pancreas between the group treated Diatarin capsules and group 2.

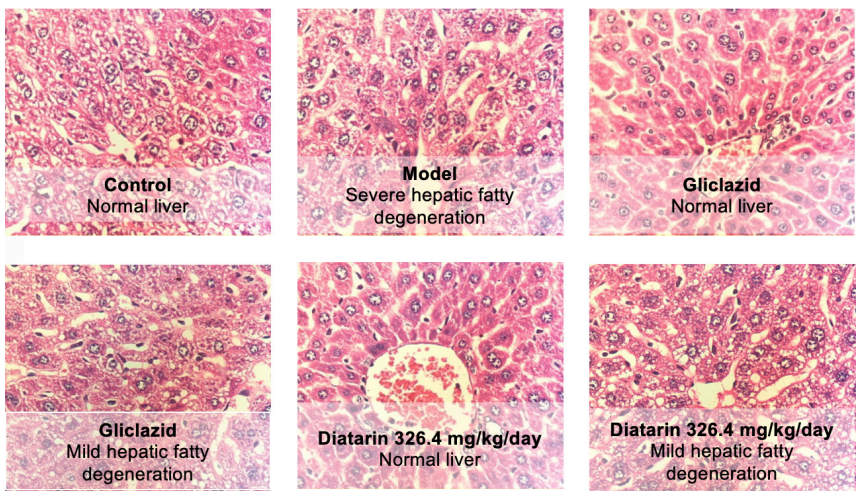

Figure 2. Micro-histopathological images of liver (HE x 400)

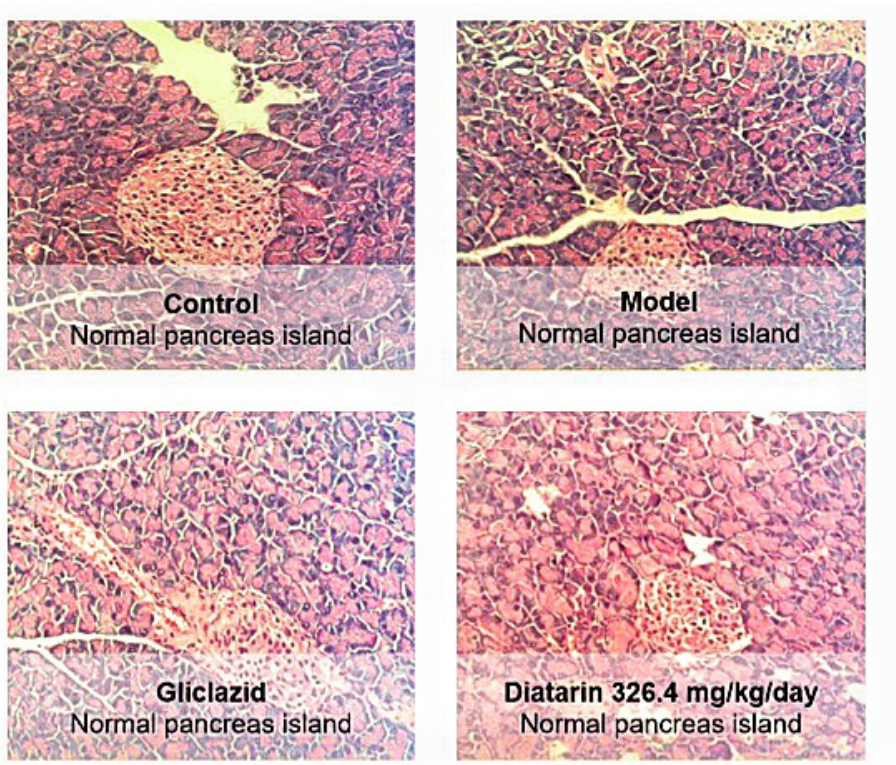

Figure 3. Micro-histopathological images of pancreas (HE x 400)

IV. DISCUSSION

The pancreas is responsible for the regulation of glucose concentrations in the plasma. Alloxan, a substance used for the induction of diabetes mellitus, possesses a destructive activity on the β-cells of the pancreas.[9] Alloxan induces the damage and death of pancreatic islet-cells in mice, thereby damaging β-cell which leads to decreased insulin secretion and hyperglycemia.[10,11] Thus, we used alloxan as the agent to induce diabetes in mice.

After 8 weeks of high fat diet (HFD), there was a statistical increase in the blood glucose level as compared with the normal fat diet (NFD) group. At 72 hours after of ALX injection, in the HFD group, the blood glucose level increased considerably and approximately 1.5 times higher than that of mice before studying. The HFD combined with a low-dose of ALX injection was proven to have effect on hyperglycemia and bring into a success in model of type 2-like diabetes.[12]

After 1 week and 2 weeks of treatment, gliclazide at 80 mg/kg/day and Diatarin capsules at 326.4 mg/kg reduced statistically blood glucose level in type 2-like diabetic mice as compared with model group.

Besides, Diatarin capsules posed the positive effect on lipid disorder conditions. Diatarin capsules at 326.4 mg/kg/day had the tendency in reducing total cholesterol and LDL-C concentration as compared with the model group.

The result of histopathological examination showed that there was a significant change in the structure of the liver after 2-week period in the group treated with Diatarin capsules.

In terms of histopathological examination of pancreas, no clear effect was observed in the group treated with Diatarin capsules as compared with the model group.

The results demonstrated the antidiabetic effect of Diatarin capsules in experimental mice fed HFD and treated with ALX at 180 mg/kg. This may be due to the effect of the main components in Diatarin capsules. The research of Tang LQ demontrated that intragastric administration of berberine (100 and 200 mg/kg) significantly decreased fasting blood glucose levels, serum content of TC, TG, LDL-C, and effectively increased HDL-C in diabetic rats.[12] A systematic review of Bule M (2019) support the hypothesis that quercetin at 10, 25 and 50 mg/kg lowers serum glucose level.[13] In the study of Niture NT (2014), administration of rutin (50 and 100 mg/kg) orally for 3 weeks treatment significantly improved body weight, reduced plasma glucose, and restored the depleted liver antioxidant status and serum lipid profile in high fat diet + streptozotocin induced type 2 diabetic rats. Rutin treatment also improved histo-architecture of beta islets and reversed hypertrophy of hepatocytes and may be useful as a diabetic modulator along with standard antidiabetic drugs.[14]

V. CONCLUSION

In light of these findings, oral administration of Diatarin capsules at the dose of 326.4 mg/kg/day for 2 consecutive weeks exhibited significant antidiabetic activity through reducing blood glucose level, plasma lipid profiles (TC and LDL-C concentration) and improving hepatocytes injury in alloxan induced type 2 diabetic model.

REFERENCES

1. International diabetes federation (IDF) Diabetes Atlas. 2019, ninth edition.

2. Katzung B.G. Chapter 41: Pancreatic Hormones & Antidiabetic Drugs. *Basic and clinical Pharmacology*, The Mc GrawHill company. 2012;743-765.

3. Cicero AFG and Tartagni E. Antidiabetic Properties of Berberine: From Cellular Pharmacology to Clinical Effects. *Hosp Pract.* 2012;40(2):56-63.

4. Zhang D, Fu M, Gao S et al. Curcumin and diabetes: a systematic review. *Evid Based Complement Alternat Med*. 2013;16 pages.

5. Ganeshpurkar A and Saluja AK. The Pharmacological Potential of Rutin. S*audi Pharm J.* 2017;25(2):149-164.

6. Bulea M, Abdurahmand A, Nikfar S et al. Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. *Food and Chemical Toxicology.* 2019;125(2019):494–502.

7. Fabiola Rivera-Ramírez, Gerardo N. Escalona-Cardoso, Leticia Garduño-Siciliano et al. Antiobesity and Hypoglycaemic Effects of Aqueous Extract of Ibervillea sonorae in Mice Fed a High-Fat Diet with Fructose. *BioMed Research International.* 2011;2011:6 pages..

8. Søs Skovsø. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *Journal of Diabetes Investigation.* 2014;5(4):349-358.

9. Escalona C.N. Rivera R.F., Garduno S.L. et al. Antiobesity and hypoglycaemic ef-

fects of aqueous extract of Ibervillea sonorae in mice fed a high fat diet with fructose. *Journal of Biomedicine and Biotechnology*. 2011;12:3-10.

10. Balamurugan K, Nishanthini A and Mohan VR. Antidiabetic and antihyperlipidaemic activity of ethanol extract of Melastoma malabathricum Linn. Leaf in alloxan induced diabetic rats. *Asian Pac J Trop Biomed 4 (Suppl 1*): S442-S448. 2014;12:3-10.

11. Nakahara Y, Ozaki K, Sano T et al. Assessment of alloxan.induced diabetic rats as a periodontal disease model using a selective cyclooxygenase (COX).2 inhibitor. *J Toxicol Pathol.* 2014;27:123-129.

12. Tang LQ, Wei W, Chen LM et al. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. *Journal of Ethnopharmacology*. 2006;108(2006):109-115.

13. Bule M, Abdurahmand A, Nikfarae S et al. Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. *Food and Chemical Toxicology.* 2019;125:494-502.

14. Niture NT, Ansari AA, Naik SR et al. Anti-hyperglycemic Activity of Rutin in Streptozotocin-Induced Diabetic Rats: An Effect Mediated Through Cytokines, Antioxidants and Lipid Biomarkers. *Indian J Exp Biol.* 2017;52(7):720-