International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 6 Issue 6 ǁ June 2017 ǁ PP.50-53

The Ability of Hydroalcoholic Extract from UrticaDioica to Suppress Di-(2-Ethylhexyl) Phthalate's Deleterious Effect on Glycemic Response in Mice

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Abstract:*Di-(2-ethylhexyl) phthalate (DEHP) is used as a plasticizer to impart flexibility during the production of polyvinyl chloride (PVC) to make medical devices, food containers, etc. Ingestion of DEHP can raise obesity and type 2 diabetes epidemics. Urticadioica (UD) has been known as a plant that decreases blood glucose. The objective of this study was to determine the effect of hydroalcoholic extract from urticadioica on glycemic response against DEHP. An interventional study with 15 adult male mice was designed. Mice were randomly assigned to 3 equal groups: control, DEHP (dose of 250 mg/kg/day), DEHP (dose of 250 mg/kg/day) plus extract of UD (dose of 100 mg/kg/day). They were treated for a period of 8 weeks. The formulated diet was common chow plus tallow (1:1). At the end, fasting blood samples were used to measure the variables. Serum glucose and insulin resistance (IR) levels of group 2 (DEHP 250 mg/kg) and group 3 (DEHP 250 mg/kg with extract of UD100 mg/kg/day) compared to the control group were increased significantly and also the insulin resistance (IR) level of group 3 compared to group 2 was decreased significantly (P < 0.05). The quantitative insulin sensitivity check index (QUICKI) levels of group 2 and group 3 compared to the control group were decreased significantly (P < 0.05). There were no significant changes in levels of serum insulin, glycated hemoglobin (HbA1c) of total blood and area under the curve in glucose tolerance test (AUC) (P > 0.05). In this study, it was shown that DEHP (250 mg/kg) impaired blood sugar control systems which can lead to pre-diabetes. We suggest that the extract of UD, by decreasing the IR level, may be useful to improve blood sugar control systems and may not be useful for reducing serum glucose level against DEHP.*
Keywords:*Di-(2-ethylhexyl) phthalate, Glycemic response, Urticadioica, Mice*

I. Introduction

Di-(2-ethylhexyl) phthalate (DEHP) improves flexibility of polyvinyl chloride (PVC). Humans are widely exposed to DEHP because it is used in many daily products including vinyl flooring, wall covering, plastic bags and covers, medical devices, food containers, cosmetics and toys (1,2,3). The most common exposure to DEHP comes through food (1) like high fat foods (dairy, poultry, and oils). Here, there are no covalent bonds between the phthalates and plastics so they can be removed by exposure to heat or with organic solvents, thus DEHP can migrate into the air, dust, water, soil, and sediment (2). DEHP is a typical endocrine-disrupting chemical (EDC), and has been reported to induce glucose intolerance and alterations in hepatic glycogen content, abnormal β-cell ultrastructure, reduced β-cell mass, and pancreatic insulin content in rats (3). In Italian adolescents, changes in DEHP metabolite levels were associated with obesity and insulin resistance (4). Some of the same researchers found that urinary DEHP metabolite levels were associated with higher fasting blood glucose levels and increased insulin resistance and were also associated with changes in serum insulin levels (5) and type 2 diabetes(6,7). A marker of oxidative stress was also higher in those with higher insulin resistance and phthalate levels (8). In animals, rats given the phthalate DEHP developed symptoms of diabetes, including higher blood sugar and lower insulin levels. The changes reversed when the exposure was removed (9).

Urticadioica, more commonly known as Nettle, is a plant where its flowers, leaves, stem, and roots have been used for medicinal purposes (10). Nettle plant includes phenolic compounds such as caffeic acid, ferulic acid, fistin, cynapic acid, scholtin, locoanthocyanidin, flavones and flavonol (11). Nettle has antioxidant activity, antiandrogenic properties, as well as anti-diabetes, antimicrobial and antiproliferative activities (12).

II. Materials and Methods

2.1. Extract preparation

Urticadioica was collected around the city of Meshkin in Iran. The leaves were dried under the shade and then ground into powder by an electrical grinder. The extraction was prepared using the maceration method.

The powder was macerated for 72 hours at room temperature using 70% ethanol and 30% water. The mixture was filtered with Whatman filter paper, and the filtrate was centrifuged at 3000 rpm for 20 mins. The supernatant was evaporated at ambient temperature. The extract powder was kept at 4˚C until used (13).

2.2. Di- (2-ethylhexyl) phthalate (DEHP)

The chemical was procured from FarabiPetrochemical Co, Iran. CAS no. 117 - 81 - 7.

2.3. Animals and diets

An interventional study with 15 NMARI adult male mice was designed with the mice weighing at 20-25 g. DEHP was dissolved in corn oil (99.5%) because this colorless viscous liquid is soluble in oil, but not in water, however, the extract from urticadioica was dissolved in water. Mice were randomly assigned to 3 equal groups of 5 each:

I) control

II) DEHP (dose of 250 mg/kg/day)

III)DEHP (dose of 250 mg/kg/day) plus extract of urticadioica (dose of 100 mg/kg/day).

They were treated for a period of 8 weeks. The formulated diet was common chow plus tallow (1:1).

2.4. Analytical methods

At the end of the seventh week study all mice were tested by oral glucose tolerance test and the area under the curve (AUC) was calculated for every sample. The mice were administered glucose (99.5%, CAS no. 50 - 99 - 7; Sigma) at a level of 0.25 g/kb body weight. Samples of whole blood (2 – 3 µl each) were collected from each mouse's tail before glucose administration with overnight fasting (16 hrs) and immediately 30, 60, 90 and 120 minutes after glucose administration (14). Blood glucose levels were measured using a Glucometer (On Call EZ,SD,USA). At the end of 16 hrs of fasting, the serum of the blood samples was separated by centrifugation at 4500 rpm for 20 mins and used to determine levels of blood glucose, Insulin, Insulin resistance (IR), the quantitative insulin sensitivity check index (QUIKI) and the total blood of samples were used to determine the level of glycated hemoglobin A1c (HbA1c). Glucose oxidase (GOD-PAP) method was used to determine serum glucose (kit glucose, Pars AzmoonCo, Iran) and serum insulin was analyzed using the Mouse Insulin ELISA kit (Cat.No&Brand: Estbiopharm, CK-E 20353, China). Insulin resistance score (HOMA-IR) was computed with the formula: fasting serum glucose (mmol/l) × fasting serum insulin (mU/l) divided by 22.5 (15) and the quantitative insulin sensitivity check index (QUICKI) was computed with the formula: 1/ (log (fasting insulin µU/mL) + log (fasting glucose mg/dL)) (16). Glycated hemoglobin (HbA1C) of 50µl total blood (EDTA used as anticoagulant) was assessed by Biosystems reagent kit provided by Biosystems, S.A. Costa Brava 30, Barcelona (SPAIN).

2.5. Statistical analysis

All data were analyzed using SPSS software and the results are presented as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Duncan's analysis test (Post Hoc) were used to compare the means, and differences were considered significant when $P \leq 0.05$.

III. Results

In the present study, it was shown that serum glucose and insulin resistance (IR) levels of group 2 (DEHP 250 mg/kg) and group 3 (DEHP 250 mg/kg with extract of urticadioica 100 mg/kg/day) compared to the control group increased significantly ($P < 0.05$) and insulin resistance (IR) levels of group 3 (DEHP 250 mg/kg with extract of UD 100 mg/kg/day) compared to group 2 (DEHP 250 mg/kg) decreased significantly ($P < 0.05$). The quantitative insulin sensitivity check index (QUICKI) level of group 2 (DEHP 250 mg/kg) and group 3 (DEHP 250 mg/kg with extract of UD 100 mg/kg/day) compared to the control group was decreased significantly ($P < 0.05$). There were no significant changes in levels of serum insulin, glycated hemoglobin (HbA1c) of total blood and area under the curve in the glucose tolerance test (AUC) ($P > 0.05$) (Table 1).

Table 1. The ability of hydroalcoholic extract from Urticadioica to suppress Di-(2-ethylhexyl) phthalate's (DEHP) deleterious effect on glycemic response in mice in mean (± SEM) biochemical parameters.

Groups	Glc mg/dL	In µIU/mL	IR	HbA1C %	QUICKI	AUC
I	61.333 ± 7.03^{b}	4.667 ± 0.203^{a}	0.70 ± 0.08^{c}	12.167 ± 1.49^{a}	0.407 ± 0.007^{b}	351.50 ± 31.19^{a}
II	102.00 ± 7.03^{a}	5.500 ± 0.203^{a}	1.37 ± 0.08^{a}	13.132 ± 1.49^{a}	0.365 ± 0.007^{a}	449.00 ± 31.19^{a}
III	68.250 ± 6.08^{a}	4.375 ± 0.176^{a}	0.75 ± 0.07^{b}	11.550 ± 1.29^{a}	0.378 ± 0.006^{a}	542.38 ± 27.02^{a}

Glc: glucose, **In:** insulin,**IR:** insulin resistance,**HbA1C:** glycated hemoglobin A1c, **QUICKI:** quantitative insulin sensitivity check index, **AUC:** area under the curve, **G-I:** Control, **G-II:** DEHP 250 mg/kg, **G-III:** DEHP 250 mg/kg plus extract of urticadioica 100 mg/kg. Each value in the table is the mean ± (SEM) (n= 5). Values in the same column sharing different letters are significantly different ($P < 0.05$) (ANOVA followed by Post Hoc).

IV. Discussion

Simultaneous intake of both a high fat diet and phthalates is a daily reality (17). That is why the formulated diet was common chow plus tallow (1:1). The objective of this study was to determine the effect of the hydroalcoholic extract from urticadioica on glycemic response against Di-(2-ethylhexyl) phthalate in mice. In this study, we used the hydroalcoholic extract from urticadioica to evaluate the influences of Di-(2-ethylhexyl) phthalate on the development of pre-diabetes. Our study showed that in DEHP-exposed mice and DEHP-exposed with extract of urticadioica mice, serum glucose levels and HOMA-IR values compared to the control group significantly increased and the quantitative insulin sensitivity check index (QUICKI) compared to the control group significantly decreased ($P < 0.05$). The quantitative insulin sensitivity check index (QUICKI) is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose and is useful for measuring insulin sensitivity (IS), which is the inverse of insulin resistance (IR) (16,18). This study showed no significant change in serum insulin level ($P > 0.05$). These results suggest that mice with the extract of urticadioica supplement did not have a well-regulated serum glucose compared to those without the extract but we suggest that the extract of urticadioica, by decreasing insulin resistance (IR) level against DEHP may be useful to improve blood sugar control systems. A previous study in mice has reported that DEHP (500 mg/kg) exposed mice with vitamin E supplement expressed high levels of plasma insulin and low levels of serum glucose and HOMA-IR value while HFD-mice with DEHP supplement expressed a much higher level of glucose with low insulin concentrations and HOMA-IR values (14). Another study showed that DEHP-exposed mice at a low dosage (7.5 mg/kg) significantly increased serum glucose levels and induced serum insulin levels as well as caused thyroid and adrenocortical dysfunction in mice (9). DEHP impaired the expression of insulin signaling molecules and their phosphorelay pathways leading to a diminished plasma membrane GLUT4 level and thus decreased glucose uptake and oxidation. It significantly decreased insulin receptor mRNA expression suggesting that DEHP downregulates the transcription of the gene and because of that DEHP decreased the level of serum insulin (19). Phthalates activate certain hormone receptors called PPARs which are known to influence blood glucose levels, via insulin resistance, as well as insulin secretion (20). In the present study, there was a no significant difference in Glycated hemoglobin (HbA1C) levels between the groups ($P > 0.05$). Phthalates may have a little effect on glycosylation of red blood cells but this should be further investigated and studied. Our study also showed non-significant changes in the area under the curves (AUC) for the glucose tolerance test ($P > 0.05$). A previous study has reported that DEHP (500 mg/kg) exposed mice fed with HFD expressed high levels of glucose at 30 and 60 minutes after glucose ingestion and low insulin concentration (14).

V. Conclusion

In this study, it was shown that DEHP (250 mg/kg) impaired blood sugar control systems and this can lead to pre-diabetes. We suggest that the extract of UD, by decreasing the IR level, may be useful to improve blood sugar control systems and may not be useful for reducing serum glucose level against DEHP.

Acknowledgement

We would like to thank the Molecular Biology Research Laboratory of the Department of Veterinary Medicine at Azad University of Tabriz for providing technical support. Also, we would like to thank the Farabi Petrochemical Co for providing the Di-(2-ethylhexylphthalate) (DEHP).

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