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Ameliorative effect of *Olax acuminata* Wall. Ex Benth. leaves aqueous extract on high fat diet induced alterations in mice

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Abstract

The present study investigates the effect of Olax acuminata *Wall. Ex Benth. leaves aqueous extract on alterations associated with high fat diet (HFD) in mice. HFD fed mice are considered as an appropriate model for obesity study. In this study, mice were fed with HFD for 45d (20g/mice/day) period. O. acuminata leaves extract (OLE; 50 mg/kg b.w.)/orlistat (ORL;10 mg/kg b.w.) was administered intraperitoneally for alternate days to HFD mice from 29d to 45d. Administration of OLE significantly improved the liver/kidney weight in mice fed with HFD. Further, treatment with OLE/ORL improved the enzyme activities i.e., hexokinase, phosphoenolpyruvate carboxykinase and acetyl coA carboxylase in liver/ kidney including the lipid profile in HFD fed mice. These findings provide evidences for the ameliorative effect of OLE against alterations observed in HFD fed mice. However, ORL-treated mice showed higher significant effect therefore, increasing the OLE dose could further improve its effect which needs further investigation.*

Keywords: High fat diet, kidney, liver, mice, *Olax acuminata,* orlistat

Introduction

Obesity is a disease that results from chronic energy imbalance and excessive accumulation of body fat. Atleast 2.8 million people are dying each year as a result of obesity (World health Organisation, June 2021). Obesity can lead to various metabolic complications such as insulin resistance, fatty liver and kidney diseases contributing to major causes of morbidity and mortality globally (Jiang *et al.*, 2016). Diet is considered as one of the major contributors to obesity (Higa *et al.*, 2014) and high fat diets (HFD) are commonly used for obesity studies in animals (Choi *et al.*, 2016; Bortolin *et al.*, 2018). In previous studies, increasing dietary fats have been shown to affect the levels of enzymes involved in carbohydrate and lipid metabolism in different tissues (Brooks and Lampi, 1996; Kume *et al.*, 2007). HFD is also reported to affect the serum lipid profile which modulates lipid metabolism (Aguilar *et al.*, 2011; Kang, 2012).

The current anti-obesity drugs often have severe adverse effects. Antiobesity drugs such as rimonabant increases the incidence of psychiatric side effects, sibutramine increases blood pressure and orlistat has gastrointestinal side effects (Padwal and Majumdar, 2007; Palacios-Martinez *et al.*, 2013; Blasio *et al.*, 2014; Krentz *et al.*, 2016). Therefore, researchers are focusing on the natural therapies in combating obesity. Herbal medicines are being used for weight control and for the treatment of obesity (Sethi, 2011). Various plant extracts have been reported to have antiobesity properties in HFD-induced obese mice (Ku *et al.*, 2012; Noh *et al.*, 2013; Li *et al.*, 2016; Liu *et al.*, 2017; Kim *et al.*, 2019; Liu *et al.*, 2019; Sheng *et al.*, 2019).

In this paper, we have investigated the effect of a medicinal plant, *Olax acuminata* Wall. Ex Benth. (Family: Olacaceae) on HFD fed mice and compared its effect with a known antiobesity drug, orlistat (ORL). *O. acuminata* (local name *dieng-tyrut*) is a shrub found in the Khasi Hills of Meghalaya and its leaves are edible (Sawian *et al.*, 2007). *O. acuminata* aqueous-methanolic leaves extract is reported to possess both hypoglycemic and an antihyperglycemic effects (Rynjah *et al*., 2016). The essential oils found in *O. accuminata* have been shown to exhibit antioxidant properties (Chetia *et al.,* 2014).

Materials and Methods

Chemicals

Adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate, cholic acid, cholesterol, malate dehydrogenase, glucose-6-phosphate, pyruvate kinase, lactate dehydrogenase were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Nicotinamide adenine dinucleotide hydrogen, phosphoenolpyruvate, uridine 5'-diphosphoglucose solution was purchased from Sisco Research Laboratory Pvt. Ltd., Mumbai, India. Whatmann filter paper No.1 was procured from GE healthcare Life Sciences, Maidstone, UK, 2-deoxy-D-Glucose, from Sigma Co.,USA and coconut oil from Marico Ltd., Puducherry, India.

Preparation of plant extract

Leaves of *O. acuminata* (Voucher no. 4975) were collected from Pomshutia, East Khasi Hills located in Meghalaya, India. Leaves of *O. acuminata* were dried at 37ºC and then powdered. Then, 100g of powdered leaves were mixed with 1000ml of distilled water for 2 h at room temperature. The mixture was filtered and the solution was evaporated to dryness using rotary evaporator (Stuart, Sttaffordshire, UK) followed by lyophiliser (Svanvac cool safe, Industry).

Yield percentage of plant extract

The yield percentage (%) for *O. acuminata* leaves aqueous extract (OLE) was calculated as per the formula given below:

$$\textbf{Yield percentage(\%)} = \frac{\textbf{Weight of extract}}{\textbf{Weight of plant material}} \textbf{ x 100}$$

Experimental model

Swiss albino mice were purchased from Pasteur Institute, Shillong. Mice were placed in suspended bracket cages housed in a room kept under controlled conditions with temperature maintained at 27-28ºC with free access to food and water. The clearance certificate for research project was approved by the Institutional Ethics Committee (IEC) guidelines of NEHU, Shillong, Meghalaya, India.

Preparation of HFD feed

The preparation of high fat diet (HFD) was prepared by mixing 2% cholesterol,1% cholic acid, 25% coconut oil and 72% grounded standard pellet diet (Nampurath *et al.*, 2008).

Administration of OLE/ORL

Mice were divided into 4 groups: (i) Normal mice fed standard food pellet, (ii) HFD control mice treated with 2% ethanol, (iii) HFD mice treated with *O. acuminata* leaves extract (OLE; 50 mg/kg b.w.) (iv) HFD mice treated with orlistat (ORL; 10 mg/kg b.w.). HFD were fed at 20g/mice/day for 45d period (Biswas *et al*., 2014). OLE/ORL was administered intraperitoneally for alternate days to HFD mice from 29d to 45d.

Determination of relative organ to body weight

The liver and kidney were carefully dissected out and weighed. The relative organ to body weight was represented as relative organ weight to body weight (g/g b.w.) of an individual mouse.

Enzyme assay

Activities of hexokinase, HK (Braithwaite *et al*., 1995), phosphoenolpyruvate carboxykinase, PEPCK (Petrescu *et al*., 1979) and acetyl Co A carboxylase, ACC (Numa *et al.,* 1971) for homogenized liver and kidney were assayed using UV-Vis spectrophotometer (CECIL CE, Cambridge, UK). Total protein was estimated as per Bradford's method using a spectrophotometer (1976).

Lipid profile measurement

Serum total cholesterol and High-density lipoprotein (HDL) cholesterol levels were measured spectrophotometrically using an assay kit (AUTOSPAN® CHOD-PAP Enzymatic End Point). Serum total triglyceride level was estimated according to the

protocol provided in the assay kit (AUTOSPAN® Liquid Gold Triglyceride GPO-PAP, End Point Assay).

Statistical analysis

The data on the effect of OLE in HFD fed mice was entered in the Microsoft® Excel spreadsheet, where it was organized and then exported to statistical software Graphpad prism for analysis. One-way ANOVA was used to test the significance among the normal control mice, HFD control mice, OLE-treated HFD mice and ORL-treated HFD mice. The values $p<0.05$*, $p<0.01$**, $p<0.001$*** were considered as statistically significant.

Results and Discussion

Yield percentage of OLE

In the present study, the yield percentage of *O. acuminata* aqueous leaves extract was found to be 7.81% ± 0.77. Earlier report showed that the yield percertage of aqueous methanolic extract of *O. acuminata* was 9.50% (Rynjah *et al.*, 2016) suggesting that the extraction yields can vary depending upon the solvent as indicated elsewhere (Nguyen *et al.*, 2022).

Effect of OLE on liver and kidney weight of HFD mice

To evaluate the effect of HFD on tissues, we measured the weight of liver and kidney after the experimental period. Our result showed significant increased in liver to bodyweight ratio and in kidney to body weight ratio in mice receiving HFD when compared to the normal control mice (Table 1). This is in support with many studies which demonstrated increased liver and kidney weight in HFD fed animal models (de Castro *et al.*, 2013; Ji *et al.*, 2017; Kim *et al.*, 2018; Feng *et al.*, 2019). HFD affects the energy balance (Oosterman *et al.*, 2015) leading to renal lipid accumulation due to insulin resistance which has been linked to declined renal function (Jiang *et al.*, 2005; Guebre-Egziabher *et al.*, 2013; Muller *et al.*, 2019). Excessive lipid accumulation observed in HFD fed animal model (Kanwal *et al.*, 2020) could have been one of the major reason for increased liver weight. Administration of extract caused significant reduction in the liver and kidney weightof HFD fed mice. The effect of the extract was as effective as that of the reference drug, ORL.

Table 1: Effect of *O. acuminata* leaves aquoeus extract on liver and kidney weight (g/g b.w.) in HFD fed mice. All data are represented as mean±SEM where n=3,*p<0.05, **p<0.01 and ***p<0.001 were considered significant.

Treatment group	Liver weight (g/g b.w.)	Kidney weight (g/g b.w.)
Normal control	0.0457± 0.0002	0.0092± 0.0002
High fat diet control (HFDC)	0.0677± 0.0018 **	0.0115± 0.0004 **
High fat diet treated with extract (50 mg/kg b.w.;OLE)	0.0597± 0.0012 ***	0.0100± 0.0000 **
High fat diet treated with Orlistat (10mg/kg b.w.; ORL)	0.0576± 0.0013 **	0.0093± 0.0003 *

Effect of OLE on HK activity of HFD mice

As shown in Table 2, The HK activity was significantly lowered in HFD control mice by 52.78% when compared to normal mice. In contrast, the HK activity in kidney was significantly increased by 53.19% in HFD control mice. HK is the first step enzyme involved in glycolysis pathway. HFD impairs glucose tolerance and hepatic insulin signaling (Peng *et al.*, 2012) thereby, hepatic HK being an insulin-dependent enzyme has been reported to be inactivated or inhibited (Ramesh *et al.*, 2017; Kim *et al.*, 2018). Kidney being insulin-independent for glucose uptake reflect its differences in their ability to metabolise glucose (Vidhya and Udayakumar, 2018) thus, could play a role in increased HK activity as observed in our study. Administration of extract significantly (**p<0.01) improved the HK activity in liver and kidney of HFD fed mice when compared to the HFD control mice. Treatment with ORL however, showed higher significant value (***p<0.01) in kidney in comparison to the OLE-treated HFD mice.

Effect of OLE on PEPCK activity of HFD mice

PEPCK catalyses the first committed step in gluconeogenesis, is thus a central player in glucose homeostasis. Significant increase (***p<0.001) in PEPCK activity was observed in liver and kidney of HFD control mice when compared to normal mice as shown in Table 2. Dysregulated glucose metabolism associated with increased gluconeogenesis and decreased glycolysis is reported to contribute to the onset of the insulin resistance (Muller *et al.*, 1997; Nordlie *et al.*, 1999). Administration of OLE markedly decreased PEPCK activity in liver (***p<0.001) and kidney (**p<0.01) of HFD fed mice as supported in previous study (Kwon and Choi, 2020). As observed in HK activity, treatment with ORL

also resulted in more significant (***p<0.001) decreasedin enzyme activity in kidney in comparison to the OLE-treated HFD mice.

Effect of OLE on ACC activity of HFD mice

ACC is the key enzyme in fatty acid synthesis and plays a critical role in lipid metabolism. A significant increase (***p<0.001) in the ACC activity was observed in liver and kidney of HFD control mice when compared to normal mice (Table 2). Inhibition of ACC has been reported to improve insulin sensitivity in mice fed with HFD (Schreurs *et al.*, 2009). In our study, HFD mice administered with OLE showed decreased enzyme activity (**p<0.01) in liver and kidney of HFD fed mice which is in line with other studies (Naowaboot and Wannasiri, 2016; Kim *et al.*, 2018). Treatment with ORL markedly decreased (***p<0.001) the enzyme activity in liver and kidney of HFD fed mice.

Table 2: Effect of *Olax acuminata* (OLE) leaves aquoeus extract on enzyme activities in liver and kidney of HFD fed mice. All data are represented as mean±SEM where n=3,*p<0.05, **p<0.01 and ***p<0.001 were considered significant. HK: Hexokinase; PEPCK: phosphoenolpyruvate carboxykinase; ACC: acetyl Co A carboxylase.

Treatment group	HK (U/mg protein)		PEPCK (U/mg protein)		ACC (U/mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal control	0.0373 ± 0.0009	0.0147 ± 0.0004	0.0057 ± 0.0007	0.0051 ± 0.0001	0.0116 ± 0.0008	0.0114 ± 0.0006
High fat diet control (HFDC)	0.0176 ± 0.0005 ***	0.0313 ± 0.0024 ***	0.0157 ± 0.0018 ***	0.0109 ± 0.0007 ***	0.0270 ± 0.0017 ***	0.0247 ± 0.0016 ***
High fat diet treated with extract (50 mg/ kg b.w.;OLE)	0.0283 ± 0.0026 **	0.0201 ± 0.0012 **	0.0083 ± 0.0003 ***	0.0077 ± 0.0003 **	0.0183 ± 0.0009 **	0.0141 ± 0.0021 **
High fat diet treated with Orlistat (10mg/ kg b.w.; ORL)	0.0295 ± 0.0023 **	0.0183 ± 0.0003 ***	0.0044 ± 0.0002 ***	0.0055 ± 0.0002 ***	0.0153 ± 0.0005 ***	0.0100 ± 0.0009 ***

Effect of OLE on lipid profile of HFD fed mice

As shown in Table 3, serum total cholesterol and total triglyceride levels were significantly (***p<0.001) increased but the HDL cholesterol level was decreased in HFD control mice when compared to normal control mice. Compared to the high-fat diet-fed mice

however, the serum total cholesterol levels and serum triglycerides were significantly (**p<0.01) reduced and the HDL cholesterol level was increased in the OLE-treated mice however, it was not found significant. Our data is supported by many studies involving plant extracts which improved the lipid profile when administered to HFD fed animal model (Kumar, 2013; Naowaboot and Wannasiri, 2016; Seyedan *et al.*, 2017; Wu *et al.*, 2020). However, in comparison to extract-treated HFD fed mice, ORL-treated mice showed more (***p<0.001) significant effect in improving the lipid profiles.

Table 3: Effect of *Olax acuminata* (OLE) leaves extract on total cholesterol level of HFD fed mice. All data are represented as mean±SEM where n=3,*p<0.05, **p<0.01 and ***p<0.001 were considered significant. NS: non-significant; HDL: High density lipoprotein.

Treatment Group	Total Cholesterol (mg/dl)	Total Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)
Normal control	81.42 ± 2.4957	95.85 ± 0.6513	52.57 ± 2.4178
High fat diet control (HFDC)	238.04 ± 4.8362 ***	154.93 ± 8.0852 ***	27.13 ± 2.8451 ***
High fat diet treated with extract (50 mg/kg b.w.; OLE)	209.94 ± 4.1378 **	120.79 ± 4.0001 **	34.68 ± 1.2678 NS
High fat diet treated with Orlistat (10mg/kg b.w.; ORL)	93.91 ± 2.0385 ***	102.76 ± 1.0633 ***	48.27 ± 0.3845 ***

Conclusion

This study revealed that at the dose of 50 mg/kg b.w. aqueous extract of *O.acuminata* showed ameliorative effect against the alterations induced in HFD fed mice. Although, the effect of the aqueous leaves extract of *O. acuminata* was not as significant as observed in ORL-treated HFD mice. Therefore, increasing the extract dose could improve its effect comparable to ORL which needs further investigation.

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