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Transgenic zero-erucic and high-oleic mustard oil improves glucose clearance rate, erythrocyte membrane docosahexaenoic acid content and reduces osmotic fragility of erythrocytes in male Syrian golden hamsters

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ABSTRACT

Brassica juncea, the Indian mustard variety has high erucic acid (22:1 n-9) in its oil, which causes several deleterious effects. The Centre for Genetic Manipulation of Crop Plants (India) has developed a zero-erucic and high-oleic acid transgenic mustard variety having 67% oleic acid, which is almost equivalent to that of olive oil, i.e. 71%. Therefore, we assessed its impact on erythrocyte osmotic fragility, fluidity and activities of membrane-bound enzymes and insulin sensitivity. 40 male Syrian golden hamsters of 6–8 weeks age, were divided into five groups, consisting of 8 hamsters in each and fed diet containing any one of the oils, i.e. groundnut (GNO), conventional mustard (OCM), low-erucic mustard (OLM), zero-erucic high-oleic transgenic mustard (OTM) and olive (OLO) at 10% level for 16 weeks. At the end, compared to OLO group, OTM-fed hamsters resisted osmotic shock-induced erythrocyte-haemolysis, which corroborated with higher docosahexaenoic acid (DHA; 22:6 n-3) levels in their erythrocyte membranes. However, it did affect neither the fluidity nor the activities of membrane-bound enzymes. Although fasting plasma glucose, insulin and free fatty acid levels were comparable among the various groups; during glucose challenge, OTM diet-fed animals displayed higher disposal rate of circulatory glucose, without altering the insulin levels, when compared to the conventional mustard; OCM. In conclusion, the consumption of oil from zero-erucic high-oleic transgenic mustard improved the DHA content of erythrocyte membrane, which possibly resisted haemolysis and enhanced glucose clearance during glucose overload. However, it did not affect the activities of erythrocyte membrane-bound enzymes and fluidity compared to olive oil.

1. Introduction

Brassica juncea or Indian mustard is a major oleiferous crop cultivated in the northern belt of India. Though people living in the northern semitropical belt of Indian sub-continent widely consume mustard oil, the presence of high amounts (50%) of erucic (22:1 n-9) is of great concern for human consumption [1]. Several reports suggest that the consumption of mustard/rapeseed oil having more than 7% erucic acid is known to cause myocardial lipidosis and fibrosis in experimental animals, thus making it undesirable for human consumption [2–4]. However, compared with other vegetable oils, mustard oil has better nutritional attributes such as low saturated fatty acid content (6.6 g/100 g), considerable amounts of linoleic (18:2 n-6 LA, 19.7 g/100 g) and α-linolenic (18:3 n-3 ALA, 9.6 g/100 g) acids, the two essential fatty acids; thus making it a good source of n-3 polyunsaturated fatty acids (PUFA) for people, who do not consume non-vegetarian foods, especially of marine origin. However, these nutritional advantages of mustard oil are curtailed by the presence of high amounts of erucic acid. Therefore, there is an absolute need to exclude erucic acid from mustard/rapeseed oil, which is achieved by genetic breeding. This has led to the development of several cultivars with improved fatty acid composition. In general, zero erucic cultivars of all Brassica species

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(*B.napus, B.juncea)* have high oleic acid (18:1 n-9) and substantial amounts of LA and ALA [4–6].

In this context, the scientists at the Centre for Genetic Manipulation of Crop Plants (CGMCP), New Delhi, India have first developed a low erucic acid variety by crossing the conventional mustard variety (Varuna) with a low erucic rapeseed variety (Heera, which is developed in Sweden however, poorly adapted to Indian agrarian conditions). To make mustard oil more suitable for Indian culinary practices (which involve frying at high temperatures) and increase its shelf life, PUFA levels, especially LA have to be reduced. For this purpose, the scientists at the CGMCP employed anti-sense RNA technology and silenced the fatty acid desaturase gene (Fad2 (1-acyl 2-oleoyl-sn glycero-3-phosphocholine-Δ12 desaturase)), which is responsible for the conversion of oleate to linoleate. This has resulted in the development of high oleate variety, with reduced linoleate content and α-linolenate content comparable to that of its parental strain. This variety is named as zero-erucic high-oleic transgenic mustard, which also has the right balance of n-6 and n-3 PUFA [7].

Among the vegetable oils, olive oil has the highest proportion of oleic acid (70%) and about 12% of LA and traces of ALA. Olive oil is one of the vital constituents of the Mediterranean diet and it has been shown to improve cardio-metabolic risk factors such as abnormal lipid profile, high blood pressure, postprandial hyperlipidemia, insulin resistance, metabolic syndrome, endothelial dysfunction and oxidative stress [8,9]. Notably, the newly developed transgenic mustard variety has 67% oleic acid in its oil [7]. Keeping this similarity in mind, the present study was aimed at assessing the impact of the oil extracted from the transgenic mustard variety, i.e. zero-erucic high-oleic acid on erythrocyte membrane physical and functional parameters and one of the components or causes of metabolic syndrome, i.e. insulin resistance in male Syrian golden hamsters and compared with the oils extracted from the conventional mustard, low erucic mustard and olive. For the first time here, we report the impact of zero erucic and high oleic mustard oil on erythrocyte osmotic fragility, erythrocyte membrane fluidity, the activities of membrane-bound enzymes and fatty acid composition in male Syrian golden hamsters.

2. Materials and methods

2.1. Chemicals, reagents and kits

All the reagents and chemicals were of analytical grade. Groundnut oil (GNO), and virgin olive oil (OLO; Leonardo) were purchased from a local supermarket. Oils from conventional mustard (OCM), low-erucic acid mustard (OLM) trans line 3.18; zero-erucic and high-oleic acid transgenic mustard (OTM) were obtained from Dr Deepak Pental (The Centre for Genetic Manipulation of Crop Plants, University of Delhi, New Delhi, India). Enzyme-linked immunosorbent assay (ELISA) kit for insulin from Merck Life Science Private Limited (Mumbai, India), and free fatty acid (FFA) assay kit from Biovision Inc. (Milpitas, California, USA) were purchased.

2.2. Fatty acid composition of the test oils

The fatty acid composition of various test oils was determined after the saponification, conversion to methyl esters, and analysis by gas chromatograph equipped with flame ionisation detector (GC-FID), according to the previously reported method. Authentic standards of various fatty acid methyl esters were run in the GC-FID and, then they were compared and identified in the test samples [10].

2.3. Study design

6-8-week-old 40 male Syrian golden hamsters (*Mesocricetus auratus*) were obtained from the National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition, Hyderabad. After acclimatisation for 15 days, the animals were divided into five groups, fed *ad libitum* on their respective diets, containing oils, *i.e.,* GNO, OCM, OLM, OTM and OLO at 10% level for 16 weeks. The fatty acid composition of various test oils and the composition of the diet (prepared according to the modified AIN93 formulae) are given in Tables 1 and 2 respectively. Animals were housed individually, in stainless steel cages with mesh floors in a room maintained at an ambient temperature of 22.0 ±1 °C, relative humidity of 50–60%, 12-h:12-h dark-light cycles and provided "humane care" in compliance with the ethical guidelines for the use of experimental animals. Prior approval from the Institutional Animal Ethics Committee (IAEC) of the NCLAS/NIN was obtained (Study No. P-36/8–2010/AV). Daily food intake and weekly body weight records were maintained for the experimental period. At the end of the experiment, the animals were fasted overnight, blood was drawn from retro-orbital sinus, using a thin-walled heparinised capillary and collected in EDTA-coated tubes (for all biochemical parameters) and heparinised tubes (for erythrocyte membrane preparation), and the animals were sacrificed by carbon dioxide asphyxiation. The animals were anaesthetised, using isoflurane (nasal inhalation) and it was ensured that they remained unconscious during the entire procedure to minimise the suffering and pain. Various tissues, such as liver and adipose tissues were collected, weighed and rapidly frozen in liquid nitrogen. The collected plasma and various tissues were aliquoted and stored at −80 °C, till further analysis.

2.4. Oral glucose tolerance test (OGTT)

Two weeks before the culmination of the experiment (*i.e.,*

Table 1
Fatty acid composition of various test oils used in the study.

Primary fatty acids (nmol %)	Groundnut oil	Conventional mustard oil	Low-erucic mustard oil	Zero-erucic high oleic transgenic mustard oil	Olive oil
Myristic acid (C14:0)	0.6	ND	ND	ND	ND
Palmitic acid (C16:0)	27.8	2.7	4.6	4.5	14.1
Palmitoleic acid (C16:1)	0.2	ND	ND	ND	ND
Stearic acid (C18:0)	4.3	1.2	2.3	1.8	2.2
Oleic acid (C18:1 n-9)	43	13.6	44.9	66.9	70.8
Linoleic acid (C18:2 n-6)	22.5	17.2	34.9	11.8	11.9
α-Linolenic acid (C18:3 n-3)	0.9	11.4	12	11	0.9
Eicosaenoic acid (C20:1)	ND	7.6	1.1	2.1	ND
Behenic acid (C22:0)	0.7	1.5	ND	ND	ND
Erucic acid (C22:1 n-9)	ND	44.7	ND	1.8	ND

ND -Non-detectable.