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Research Paper

Dose Dependent Effect of Olive Leaves Extract on Inflammatory Cyclooxygenase (COX) Levels in High Fat Diet (HFD) Induced Diabetic Animals

Medical Science

KEYWORDS : Cyclooxygenase (COX), Olive Leaf Extract (OLE), HFD- High fat diet.

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ABSTRACT

Background : Development of diabetes and its complications are associated with persistent inflammatory activity. It has been suggested that COX-mediated pathway involved in this inflammatory process in diabetes. There is a scarcity of the studies on anti-inflammatory effect of olive leaf extract so we have chosen the Olive leaf extract for this study.

Materials and methods: Albino Wistar rats were divided into five groups. 1. Control, 2.Control + Olive Leaf extract, 3.High fat diet induced Diabetes, 4.HFD + Olive leaf extract (100mg/kg), 5.HFD + Olive leaf extract (200 mg/kg). Whole blood sample was collected after 9 weeks of experimental period and analysed.

Results: Increased COX I expression in platelets & COX II expression in monocytes were observed in HFD diabetic animals when compared with control animals. However, olive leaf extract treatment to HFD induced diabetic animals restored the aforementioned parameters to normal level.

Conclusion: To the best of our knowledge we have done the first research on anti-inflammatory & hypolipidemic activity of Olive leaf extract and we proved it.

Introduction

The incidence of type 2 diabetes is rapidly increasing in all parts of the world. The pathogenesis of type 2 diabetes is not understood in any great detail, but it is generally believed that insulin resistance in skeletal muscle and adipose tissue is the early event (Pessin et al., 2000). Previous reports have shown obesity as a primary contributor to the acquired insulin resistance (Bogardus et al., 1985). Numerous studies have documented the development of insulin resistance as a result of increased intake of dietary fat (Kraegen et al., 1986). An impaired ability of insulin to stimulate glucose uptake in skeletal muscle with high fat feeding has been demonstrated in both invitro and invivo preparations (Bernard et al., 1997). In both humans and animals there is an inverse relationship between fasting plasma triglyceride level and insulin sensitivity (McGarry et al., 2001). There is even stronger relationship between the accumulation of intracellular triglycerides and insulin resistance. Taken together, these data implicate increased dietary fat as a causative link between obesity, insulin resistance and development of type 2 diabetes complications.

Olive leaves and olive oil in the Mediterranean diet have been the focus of many epidemiological studies and have been shown to reduce the incidence of heart disease (Tutour et al., 2002). In vitro, Olive leave extract- major metabolites Hydroxytyrosol and tyrosol provides resistance to oxidation (Visioli et al., 2002) and also it exhibited a range of pharmacological properties beneficial for the cardiovascular system. These actions included enhanced nitric oxide production by mouse macrophages (Visioli et al., 1998) protection against oxidative myocardial injury induced by ischemia and reperfusion (Manna C et al., 2004) decreased blood pressure, inhibition of platelet aggregation. The Lyon Diet Heart Study tested a Mediterranean-type diet over long term and suggested that a comprehensive strategy to decrease cardiovascular morbidity and mortality should include primarily a cardio protective diet (Lorgeril et al., 1999). There is a growing interest in the use of natural antioxidants as bioactive components in food, and such foods have been termed "functional foods" (Hertog et al., 1993). Due to their ability to scavenge ROS, antioxidants are capable of inhibiting the process of LDL cholesterol oxidation subsequently decreasing the risk of cardiovascular diseases (Jemai et al., 2008). Although oxidation of LDL can be prevented by the addition of synthetic antioxidants, greater attention is now focused on natural antioxidants because of their better safety compared to that of synthetic compounds (Al-Azzawie et al., 2006). The protective effect of these diets, which are rich in fruits and vegetables, against coronary heart disease and certain cancers have been attributed partly to the antioxidants found in them, particularly to polyphenols (Hertog et al., 1993). In the current study, we used extract of Olea europaea L. leaves to determine the effect of olive leaf extract on COX mediated pathway in high fat diet induced diabetic animal models.

Materials & Methods

Male Wistar rats weighing 200-300 grams were used for this study. Animals were obtained from the institute central animal house and maintained in standard laboratory conditions at 22 + 20 C with 12:12 hours dark-light cycle in the department animal room facility. All experimental procedures used in this study were approved by the Institute Animal Experimentation Ethics Committee. Animals were allowed to acclimatize for one week to departmental animal room environment and then randomly assigned to the following four groups.

Group 1. Control – Rats fed with rodent chow.

Group 2. Control + OL – Rats fed with rodent chow + extract of Olive Leaf (100mg/kg)

Group 3. High fat diet – Rats fed with high fat diet.

Group 4. High fat diet + extract of Olive Leaf – Rats fed with high fat diet supplemented with extract of Olive Leaf (100 mg/kg)

5. HFD + extract of Olive Leaf – Rats fed with high fat diet supplemented with extract of Olive Leaf (200 mg/kg).

Samples were collected from Male Wistar rats were anesthetized with carbon dioxide followed by decapitation. Whole blood (approximately 10 mL) was collected by draining the blood from the neck of the rats into a centrifuge tube containing citrate buffer (129 mM sodium citrate, pH 7.4) as anticoagulant (1 part citrate buffer: 9 parts blood). The collected blood sample was divided equally for platelet or monocyte isolation.

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Western blot: COX protein The density of all Western blot bands was analyzed using the Chemi Doc™ XRS system (Biorad, CA, USA).

Results

S.NO	Parameter	Group I Control (n=8)	Group II Control +OL (n=8)	Group III HFD (n=8)	Group IV HFD + OL (n=8)
1	Body Weight (gms)	418.6 ± 1.8	401.2 ± 1.9	476.0 ± 1.4**	424.0 ± 1.0**^
2	Glucose (mg/dl)	84.5 ± 0.3	82.9 ± 0.4	126.6 ± 0.5**	100.8 ± 0.5**^
3	Insulin (µU/ml)	26.0 ± 1.50	22.3 ± 1.11	43.2± 1.09**	33.4 ± 1.17**
4	HOMA-IR	4.8 ± 0.6	4.6 ± 1.05	8.6 ± 1.2**	5.1 ± 1.3**
5	Total Cholesterol (mg/dl)	28.1 ± 0.1	25.1 ± 0.1	37.8 ± 0.2**	31.8 ± 0.2**^
6	Triglycerides (mg/dl)	5.4 ± 1.1	4.8 ± 1.5	19.8 ± 0.3**	10.5 ± 1.0**^
7	Plasma MDA (micromoles/L)	2.88 ± 0.48	2.53 ± 0.44	4.30 ± 0.31**	3.01 ± 0.10^

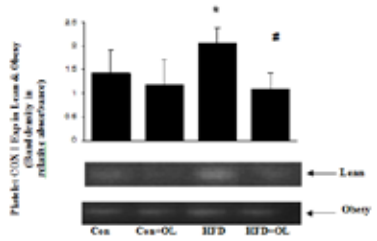


Figure 1. Effect of HFD and Olive leaf extract supplementation on Platelet COX I expression in lean & obesy Wistar rat

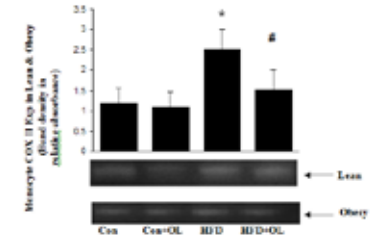
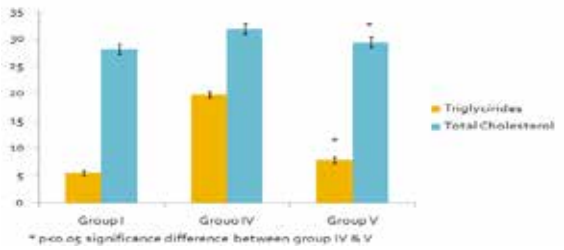


Figure 2. Effect of HFD and Olive leaf extract supplementation on Monocyte COX II expression in lean & obesy Wistar rat

Quantitative densitometry of COX proteins in Wistar rats
Panel 1: Platelet COX-1 protein expression was measured in lean and obese Wistar rats.

Panel 2: Monocyte COX-2 protein expression was measured in lean and obese Wister rats. A rat platelet or monocyte standard control was run with each Western blot and the density of all sample bands were expressed as a percentage of the corresponding standard control. In all cases, 10 µg of total protein of platelet or monocyte samples was used.

Values represent the mean, S.E. and number of Wistar rats. * Significant difference from lean values, p<0.001, unpaired Student's t-test; # significant difference from Obese values, p<0.05; ^ significant difference from Obese values p<0.05; ^ significant difference from Obese values p<0.001. Mann Whitney test.



Panel 3: Lipid profile was measured in Group I (Control), Group I V(HFD +OLE 100mg/KG) and Group V(HFD +OLE 200mg/KG) Wister rats.

Discussion
The question of this current study was effect of olive leaf extract on, COX mediated pathway in diet induced animal models. The animal model used was obese Wistar rats, which has several of the elements of Type 2 diabetes including hyperglycaemia, obesity, dyslipidemia and hypertension. In the present study, COX-1 levels in platelets were measured in obese Wistar rats and found to be elevated compared to lean Wistar rats. Similar finding has also been reported by Raju et al & Satyanarayana et al.

A study by Wang L proposed that animals in the high-lipid diet group had higher levels of cholesterol, triglycerides, and LDL cholesterol, as well as a thick layer of lipid disposition in the aortic intima compared to those in the OLE group. In our study also it has been proved and we elucidated the anti inflammatory effect is due to suppression of COX I & II respectively in platelets and monocytes. These results support olive leaf's anti-atherosclerotic effect, most likely related to suppression of inflammation.

Andreadou reported that, lipid lowering action of phenolic compounds & flavonoids decreased the blood lipid concentrations could be due to agonist actions on bile acid activated metabotropic G-protein–coupled receptor which increase in intracellular activation of thyroid hormone, hence preventing the development of obesity and insulin resistance in rat fed a high-fat diet. We also found the lipid lowering action of OLE. When we increased the OLE dose form 100mg/kg to 200mg/kg for high fat diet induced diabetic animals, it restored the aforementioned lipid profile parameters to normal level.

A research study by Gonzalez M, demonstrated OLE has a beneficial effect on blood sugar levels in animals. In rats with alloxan-induced diabetes, decreased blood glucose values significantly and increased peripheral glucose uptake. Its luteolin and oleanolic acid constituents have also been shown to have an inhibitory effect on postprandial glucose increase in diabetic rats.

Animal studies demonstrate OLE given to hypertensive rats at dosages ranging from 100-1,000 mg/kg for 2-6 weeks significantly lowered mean arterial pressure and heart rate.[4,5] Another animal study showed OLE given to salt-sensitive, genetically hypertensive rats at 60 mg/kg body weight for six weeks prevented the development of severe hypertension and atherosclerosis and improved insulin resistance (Perrinjaquet et al., 2008). Our results also showed the dose dependent variation in the fasting glucose, Insulin &HOMAIR levels.

Conclusion:- In this study, we have proved the anti-inflammatory activity of olive leaf extract by inhibiting the COX. It is also been proved that the higher dosage (200mg/kg) form it will restore the aforementioned lipid profile parameters to normal level. Polyphenols rich olive leaf extracts are safe and present potent anti- inflammatory and antioxidant effects. Olive leaf by-products generated can be used beneficially as development of new drugs of anti inflammation

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