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RESEARCH ARTICLE

EFFECT OF THE HYDROALCOHOLIC EXTRACT OF *OLEA EUROPEA* LEAVES ON GLUCOSE TOLERANCE AND PLASMA LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC RATS.

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Abstract

This work was conducted to study the effects of the hydroalcoholic extract of olive (*Olea europea* L) leaves on glucose tolerance and plasma lipids level in alloxan-induced diabetic rats. Two experiments were carried out, in the first one, the diabetic rats were divided into three groups (8 rats each) namely; control group which received normal saline, reference group which received glipiside (an antidiabetic drug) and extract group which received the hydroalcoholic extract of olive leaves (400 mg/Kg b.w.) and their blood sugar levels were determined every 2 hours. In a long term experiment, the diabetic rats were divided randomly into two groups (8 rats each), one group received daily the hydroalcoholic extract of olive leaves in a dose of 400 mg/Kg b.w. dissolved in 1.0 ml normal saline and the second group (diabetic control) received normal saline only (1.0 ml) by using stomach tube during the experiment period. Oral glucose tolerance test was evaluated weekly during the experiment period, in addition to insulin levels and lipid profile. The results revealed that the hydroalcoholic extract of olive leaves improved the glucose tolerance (by decreasing the area under the plasma glucose concentration time curves (AUC); lowered the fasting plasma glucose (FPG) level and increased insulin secretion in the extract-treated group as compared to control. Also, the obtained results showed significant reductions in total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-c) levels in the plasma of the extract-treated group when compared with the control group. This study demonstrated that intake of olive leaves or its extract, either in a tea drink form or by adding it to the food, has beneficial effects on glucose tolerance and lipid metabolism especially in individuals with non-insulin dependent diabetes (NIDDM).

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Introduction:-

Diabetes mellitus is a group of metabolic disorders in which the main character is hyperglycemia. It affects nearly 10% of the world population (Burke et al., 2003) and is the major cause of morbidity in developed countries (Ravi et al., 2005). Hyperglycemia which is associated with disturbances in carbohydrates, fat and protein metabolism is resulting from defects in insulin secretion or insulin action (Sharma et al., 2008). The aim of diabetes treatment is to achieve normoglycemia to prevent later cardiovascular complications such as retinopathy,

nephropathy, neuropathy and microangiopathy. Since lipid abnormalities lead to premature atherosclerosis, the major cause of cardiovascular (CV) diseases in diabetic patients, the ideal treatment of hyperglycemia and diabetes should improve lipid profile. Most types of the oral antidiabetic drugs used in treatment of type 2 diabetes do not have a favorable effect on CV diseases (Fisman et al., 2008). Therefore, it is important to look for new non-toxic therapeutic agents to treat diabetes which also should correct dyslipidemia to reduce the risk of CV complications of diabetes. A large number of plants have been used in the traditional medicine for treatment of diabetes (Marles and Farnsworth, 1994; Jouad et al., 2001; Bnouham et al., 2002; Tahraoui et al., 2007).

Olive tree (*Olea europea* L., *Oleaceae*) is a long lived evergreen tree that is widespread in various parts of the world. Olive leaves have been widely used in traditional remedies in European and Mediterranean countries (Bennani-Kabchi et al., 1999). They have been used in the human diet as herbal teas and powder in addition to their extracts where they contain several potentially bioactive compounds that may have antioxidant, antihypertensive, anti-atherogenic, anti-inflammatory, hypoglycemic and hypocholesterolemic properties (Sedef and Karakaya, 2009). The bioactivity of olive leaves extracts appears to be attributable to their phenolic compounds such as oleuropein, hydroxytyrosol, oleuropein aglycone and tyrosol (Visioli et al., 2002). Several studies have shown that oleuropein which represents about 8% of the dry leaves possesses an antihyperglycemic effect in diabetic rats (Gonzalez et al., 1992; Jemai et al., 2009). The hypoglycemic and antioxidant effects of oleuropein have been reported in alloxan-diabetic rabbits (Al-Azzawie and Alhamdani, 2006; Benhabyles et al., 2015). Wainstein et al. (2012) reported that the alcoholic extract of olive leaves improved glucose homeostasis in individuals with diabetes. This study was carried out to evaluate the antihyperglycemic effect of the hydroalcoholic extract of olive leaves by assessing blood glucose and insulin levels and glucose tolerance in alloxan-induced diabetic rats. Since diabetes is often accompanied by hyperlipidemia, the effect of the extract on lipid profile of the examined rats was also investigated.

Materials and methods:-

Plant material:-

The leaves of *Olea europaea* L were obtained from Al-Farafra Oasis, West of Egypt. The leaves were allowed to air dry at room temperature and then ground. The obtained powder was kept in a dark glass bottle and stored in the freezer till use.

Preparation of the olive leaves extract:-

About 500 grams of olive leaves powder were soaked in 3 liters of ethanol overnight at room temperature, and then filtered and the ethanolic filtrate was collected. Another portion of ethanol was added to the plant residue and boiled for two hours under reflux condenser, then filtered and the filtrate was added to the previous extract. In the same manner, 2 liters of distilled water were added to the plant residue and left at room temperature overnight, then filtered and the filtrate was added to the collected extract. Another volume of distilled water was added to the residue and boiled for two hours under reflux, then filtered and the filtrate was added to the previously gathered extract to form the hydro-alcoholic extract. The solvents were distilled by using rotary evaporator to get the hydroalcoholic extract which was washed three times by petroleum ether, then dried and kept in dark bottles under freezing until use.

Animals:-

Male rats, 7 weeks old (weighting 200 – 250 g) were obtained from the Faculty of veterinary medicine, Cairo University. The rats were housed in stainless steel wire cages in an air conditioned room (25°C with 55% humidity) under a 12 h light and 12 h dark cycle in the animal house of Mansoura University, college of Medicine. The animals were fed on a commercial diet and water provided *ad libitum*.

Induction of diabetes in rats:-

Diabetes was experimentally induced by a single intraperitoneal injection of a freshly prepared alloxan monohydrate (120 mg/Kg b.wt. in normal saline) to overnight-fasted rats (Tank *et al.*, 1989). After 5 days only those rats which showed plasma glucose levels above 200 mg/dl were classified as diabetic and were used in the study.

Experiments:-

Two experiments were carried out in this study, one short-term and the other is long-term experiment.

The short-term experiment:-

In the short-term experiment, three groups of diabetic animals were used (8 rats each), these were control group, reference group and the extract group. All the rats were fasted for 12 hours and their fasting blood glucose levels were determined, and then treated by intragastric tube as follows:

1. Diabetic control group: received normal saline only (1.0 ml),
2. Reference group: treated with glipiside (an antidiabetic drug) in a dose of 2.5 mg/kg b.wt. in normal saline.
3. The extract group which treated with the hydroalcoholic extract of olive leaves (400mg/kg b.wt.) in normal saline.

After administration, blood samples were collected at 2, 4 and 6 hours and their plasma glucose levels were estimated.

The long-term experiment:-

In the long-term experiment, the diabetic rats were divided into two groups of untreated and treated (each of them with 8 rats). The untreated group, which served as a diabetic control, was orally administered normal saline (1.0 ml daily) and the other group (extract-treated group) was orally administered the hydroalcoholic extract of olive leaves (400 mg/Kg b.wt. in normal saline) through an intragastric tube every day for 6 weeks. Fasting plasma glucose, cholesterol, triglyceride and HDL-c levels were determined once a week at specific time. Oral glucose tolerance test (OGTT) was performed every week in addition to blood insulin levels.

Collection of blood samples:-

Blood samples were taken from the orbital venous plexus of rats by using hematocrit tubes under ether anesthesia and collected in heparinized tubes, then centrifuged for 10 min at 4000 rpm for plasma separation. Plasma samples were stored in deep freezer till analyses.

Oral glucose tolerance test:-

An oral glucose tolerance test (OGTT) was performed every week. All rats were fasted for 12 hours before OGTT. Glucose (2 g/Kg b.wt.) was administered orally to each rat and the blood glucose and insulin levels were measured before the treatment and every 30 minutes for 2 hours of treatment. The areas under the glucose curves were calculated by using the following formula: $AUC = 0.25 \times (\text{fasting value}) + 0.5 \times (\text{half-hour value}) + 0.75 \times (\text{1-hour value}) + 0.5 \times (\text{2-hour value})$ as described by Haffner *et al.* (1986).

Insulinogenic index:-

An insulinogenic index defined as the ratio of the change in circulating insulin to the change in the corresponding glycemic stimulus (Seltzer *et al.*, 1967). It was calculated by using the equation: $\{30\text{-min plasma insulin} - \text{fasting plasma insulin} (\mu\text{U/ml})\} \div \{30\text{-min plasma glucose} - \text{fasting plasma glucose (mmol/l)}\}$.

Biochemical analyses:-

Glucose, total cholesterol and triglyceride (TG) levels were measured in blood plasma by using enzymatic colorimetric methods (bio-Merieux Kits) according to the methods of Siest *et al.* (1981), Deeg and Ziegenhorn (1982) and Trinder (1969), respectively. HDL-cholesterol was determined by using Diamond Kit according to Richmond (1973).

LDL-cholesterol was calculated by the following formula,

$$\text{LDL-cholesterol (mg/dl)} = \text{Total cholesterol} - \text{TG}/5 - \text{HDL-cholesterol}.$$

Plasma insulin levels were determined according to the method of Hoier and Jensen (1993) by using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis:-

Results were presented as the mean \pm standard error (SE). A one-way analysis of variance (ANOVA) was performed using SPSS-15 software and the significance calculated using student's "t" test. The values were considered significantly different when the p value was lower than 0.05.

Results and discussion:-

Effect of oral single dose of the hydroalcoholic extract of olive leaves on blood glucose levels in diabetic rats:-

The data in Table (1) revealed that the hydroalcoholic extract of *Olea europea* has a significant hypoglycemic effect on alloxanized rats as compared to diabetic control ($P < 0.05$). The reductions in blood glucose were 27, 34 and 36% at 2, 4 and 6 hours after administration, respectively. It was noticed that the hypoglycemic effect of the olive leaves extract was more effective than that observed with glipiside, the antidiabetic drug which exhibited a maximum reduction of 23% at 4 hours after treatment.

Table 1:- Effect of the hydroalcoholic extract of olive leaves on fasting blood glucose levels in alloxan-induced diabetic rats.

Treated groups	Relative blood glucose levels						
	Zero time (a)	Time after orally administration					
		2 hrs.		4 hrs.		6 hrs.	
		mean	% (b)	mean	%	mean	%
Diabetic Control (Distilled water)	100	106±6	100	100±5	100	104±8	100
Reference (Glipiside, 2.5mg/kg)	100	94±7*	89	79±9*	79	80±10*	77
Olive extract (400mg/kg b.w.)	100	77±6*	73	66±8*	66	67±12*	64

The values are expressed as the mean ± SE of the mean of eight rats in each group.

(a) Fasting blood glucose level at zero time: 200 – 300 mg/dl

(b) The results of treatments were calculated as percentage from control

* Significantly different from the control values of diabetic rats at $P < 0.05$.

Effect of the hydroalcoholic extract of olive leaves on oral glucose tolerance test (OGTT) and insulinogenic index in alloxanized rats:-

In a long-term study, the diabetic rats were subjected weekly to oral glucose tolerance tests to evaluate the glucose regulatory capacity. The results of OGTT curves illustrated in Fig.1 and Area under the plasma glucose concentration curve (AUC) in Table (2) showed no significant difference in the first week between the control group and the treated group, which received 400mg/kg of olive leaves extract. From week two to week six, the diabetic animals which had been treated with olive extract showed a significant improvement in glucose tolerance as compared to diabetic control. A significant reduction in plasma glucose level was observed in the extract-treated group at 30 and 60 minutes after glucose load in comparing with the control group as shown in all the curves (Fig. 1). It was also noticed that the area under curve (AUC) in the extract-treated rats was significantly lower than that of the control group from week two to week six ($P < 0.05$). It was clear that the olive leaves extract revealed a gradual improvement in the glucose tolerance during the experiment period as illustrated in the glucose tolerance curves (Fig.1). The reduction percent in the AUC reached 22% in the fifth week and 20% in the sixth week.

Effect of olive leaves extract on fasting plasma glucose level and insulinogenic index (II) of the diabetic rats during the experiment period:-

The effect of olive leaves extract on plasma glucose level in diabetic rats was studied for 6 weeks and the results were recorded in Table 2. The results showed no significant difference in fasting plasma glucose level between the control group and extract-treated group in the first week. From the second week to the sixth week, the fasting plasma glucose level in rats treated daily with the hydroalcoholic extract of olive leaves (400mg/kg) was significantly lower in comparing with that of the control group. The maximum reduction in fasting glucose level reached 20% after 6 weeks of extract administration to the diabetic rats when compared with control group. These findings indicate that the extract of olive leaves in addition to its antihyperglycemic activity, improves the diabetic patient status in the long run.

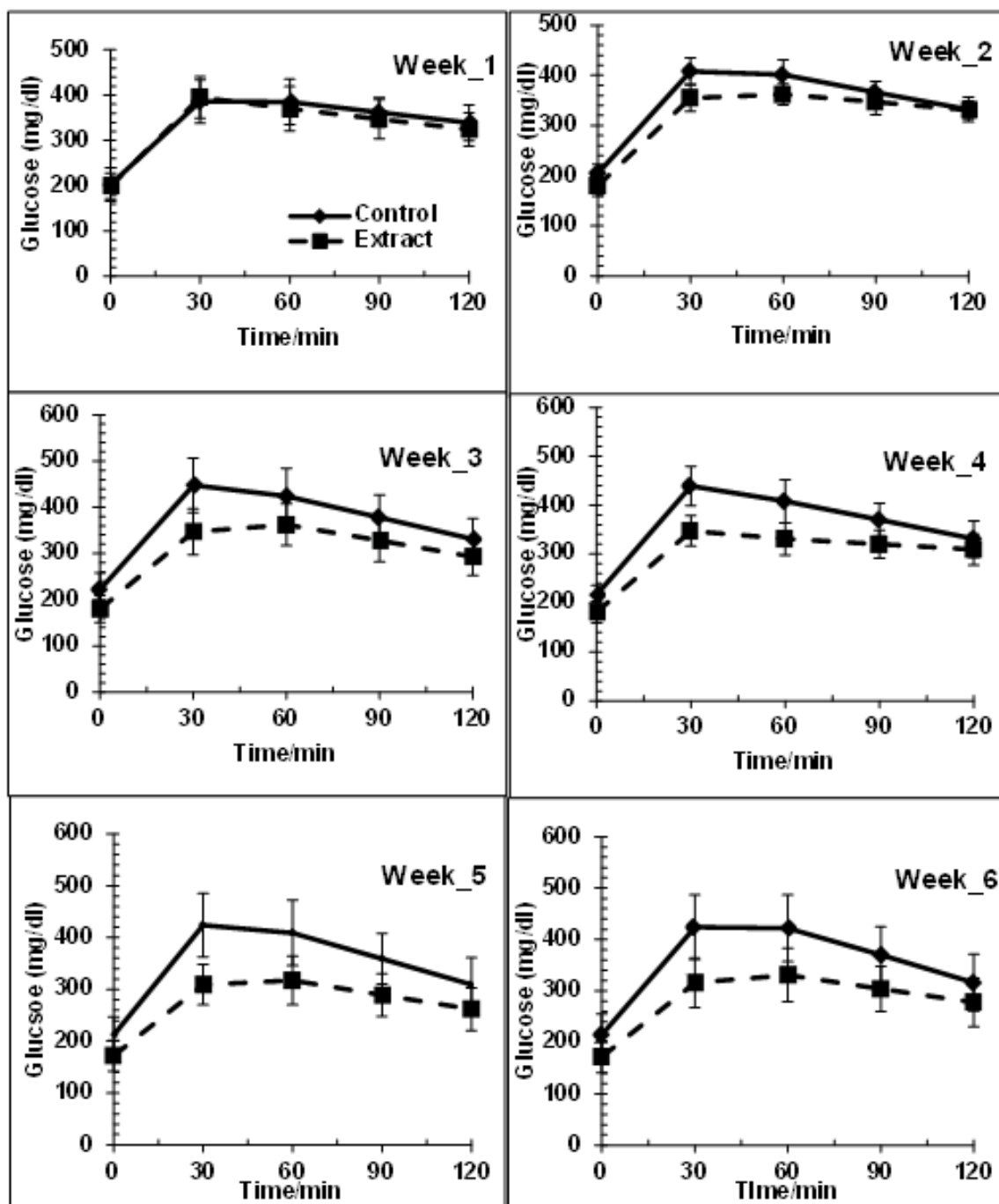


Fig 1. Glucose tolerance curves for the diabetic control group (—◆—) and the extract group (—■—) which was treated with the hydroalcoholic extract of olive leaves

On the other hand, a significant difference in the insulinogenic index (IGI) between the olive extract-treated group and the control group was noticed from the second week to the sixth one (Table 2). The insulinogenic index (IGI) is a frequently used index of β -cell function. It is an index of insulin secretion derived from OGTT (Goedecke et al., 2009). The significant increase in insulinogenic index began from the second week and reached its maximum at weeks 4, 5 and 6. This result indicates that the olive hydroalcoholic extract has the potentiality to increase plasma insulin levels either by increasing its secretion from pancreatic beta cells or by releasing it from the bound form.

Table 2:- Effect of the hydroalcoholic extract of olive leaves on fasting plasma glucose (FPG), Area under the plasma glucose concentration time curve (AUC) and insulinogenic index (IGI) values in alloxan-diabetic rats.

	Before treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
FPG (mg/dL)							
Control	209 \pm 20	208 \pm 10	213 \pm 14	222 \pm 21	216 \pm 17	212 \pm 22	215 \pm 21
Olive extract	206 \pm 18	200 \pm 11	180 \pm 12*	180 \pm 19*	181 \pm 13*	172 \pm 19*	172 \pm 17*
AUC(mg/dl min)							
Diabetic control	722 \pm 55	702 \pm 44	722 \pm 25	763 \pm 54	745 \pm 38	726 \pm 56	740 \pm 61
Olive extract	715 \pm 31	687 \pm 35	659 \pm 27*	636 \pm 43*	621 \pm 30*	566 \pm 39*	588 \pm 44*
IGI (uU/mM/L)							
Diabetic control	4.38 \pm 1.1	4.62 \pm 0.9	4.31 \pm 1.0	4.46 \pm 0.6	4.45 \pm 0.8	4.71 \pm 0.7	4.83 \pm 1.1
Olive extract	4.43 \pm 1.0	5.25 \pm 0.9	6.42 \pm 0.8*	7.51 \pm 0.9*	7.74 \pm 1.1*	7.83 \pm 1.2*	7.94 \pm 1.3*

The values are expressed as the mean \pm SE of the mean of eight rats in each group.

* Significantly different from the control values of diabetic rats at $P < 0.05$.

Effect of the hydroalcoholic extract of olive leaves on lipid profile of alloxan-diabetic rats:-

From Table 3, it was obvious that the hydroalcoholic extract of olive leaves caused a significant decrease in lipid profile parameters, except HDL-c in the diabetic rats. Plasma total cholesterol level of the rats received olive extract was lower than that of the control group from the first week to the end of the experiment. The maximum reduction in total cholesterol reached 31.3% at the fifth week after extract administration when compared with control group.

The results also showed significant reduction in plasma triglyceride (TG) concentration of the extract-treated group when compared with control group during the period of the experiment. The maximum reduction in plasma TG was 33.6%, which registered on the 5th week

On the other hand no significant changes in the plasma HDL-cholesterol levels were observed in the extract-treated group when compared with control group (Table 3). The results also revealed significant decreases in the plasma LDL-c (bad cholesterol) levels of the extract-treated group in comparison with control group. The maximum reduction in Plasma LDL-c level of the extract-treated group reached 45%. It was also noticed that HTR% (HDL-c / Total Cholesterol Ratio) in the extract-treated group was 43.2% versus 31.4% for control group at the week five. These results agree with that obtained by Eidi et al. (2009) and Somova et al. (2003) who reported that *Olea europea* has hypolipidemic effects in diabetic rats.

Other studies support the fact that olive leaves extracts are rich in oleuropein and hydroxytyrosol compounds, to which the antioxidant activities of olive leaves are attributed. Thus these phenolic compounds could prove to be beneficial in the protection against metabolic diseases associated with oxidative stress such as diabetes. In our study, the use of the hydroalcoholic extract of olive leaves revealed a strong antidiabetic effect on alloxan diabetic rats either in short-term study or long-term one. The antihyperglycemic effect of the hydroalcoholic extract of olive leaves has been confirmed by decreasing the area under glucose tolerance curve and increasing insulin secretion as indicated by increasing of insuligenic index with the duration of the treatment.

Table (3):- Effect of the hydroalcoholic extract of olive leaves on plasma levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in alloxan-induced diabetic rats.

Treatment	Before	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
TC (mg/dL)							
Diabetic control	130+21	142+ 16	153+17	170+18	182+21	179+22	182+18
Olive extract	123+18	125+15*	126+16*	129+15*	128+14*	123+16*	130+17*
TG (mg/dL)							
Diabetic control	85+16	97+ 16	100+17	103+19	111+17	116+18	103+17
Olive extract	83+13	75+10*	80+17*	84+18*	82+16*	77+16*	78+10*
HDL-c (mg/dL)							
Diabetic control	52.8+11	51.4+ 10	52.8+12	54.2+11	57.0+12	56.3+11	56.7+12
Olive extract	50.9+12	50.4+10	50.5+9	52.0+11	54.7+10	53.1+10	51.7+11
LDL-c (mg/dL)							
Diabetic control	60.2+9	71.2+ 8	80.2+9	95.2+11	102.8+11	99.5+11	104.7+12
Olive extract	55.5+8	59.6+7	59.5+8*	60.2+8*	56.9+7*	54.5+8*	62.7+10*

The values are expressed as the mean \pm SE of the mean of eight rats in each group.

* Significantly different from the control values of diabetic rats at $P < 0.05$.

Our results are in agreement with other findings on the antihyperglycemic effect of olive leaf extract (Jemai et al., 2009; Cumaoglu et al., 2011). De Bock et al. (2013) reported that the polyphenols of olive leaves improved insulin sensitivity in middle-aged overweight men. They found that supplementation of diet with olive polyphenol extract for twelve weeks caused 15% improvement in insulin sensitivity and 28% increase in β -cell function of the test group compared to placebo. This implies that olive leaf can ameliorate insulin resistance in overweight subjects

The pathophysiology of diabetes clearly revealed a link between diabetes and dyslipidemia, which characterized by high levels of total cholesterol, low density lipoprotein (LDL-c) and triglycerides (Goldberg, 2001). This is the cause of the higher incidence of cardiovascular diseases in patients with type 2 diabetes than that of non-diabetics.

In the present study, the hydroalcoholic extract of olive leaves showed a significant decrease in total cholesterol, triglycerides, low density lipoprotein in comparing with diabetic control while high density lipoprotein (HDL-c) did not affect. Fki et al. (2007) reported a hypolipidemic effect of *Olea europaea* in diabetic rats which have been attributed it to the polyphenols, oleuropein and hydroxytyrosol in the olive by-products (Jemai et al., 2008; Somova et al., 2003). In agreement with the present findings, other reports showed hypoglycemic and hypocholesterolemic effects of olive leaves (Verspohl, 2002; Jouad et al., 2001; Alarcon-Aguilar et al., 2002).

They also found that the atherosclerotic index, the ratio of LDL-c and HDL-c, was significantly lower in the groups that were administered olive leaf extracts ($p < 0.05$). Vogel et al. (2015) found that the phenolic compounds present in olive leaves, especially the oleuropein, are associated to antioxidant, antihypertensive, hypoglycemic, hypocholesterolemic and cardioprotective activity.

It is clear from these results that olive leaves extract is very useful for diabetic patients with hyperlipidemia and those who are suffering from cardiovascular diseases.

Conclusion:-

This study showed that olive leaves contain effective substances which help in reducing the levels of blood glucose and improved the diabetic status in the experimental animals as indicated by improving their glucose tolerance and increasing the secretion of insulin. The results also showed that the hydroalcoholic extract of olive leaves significantly decreased the blood levels of cholesterol, triglycerides and LDL-c which makes it helpful to the patients of cardiovascular diseases. However, further studies need to be conducted to elucidate the bioactive components and their mechanism of action.

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