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EFFECT OF OLIVE OIL ON INSULIN RELEASE, INSULIN RESISTANCE AND LIPID PROFILE AND ITS INTERACTION WITH METFORMIN IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

In the present study, effect of olive oil and its interaction with metformin, a well-known oral hypoglycemic agent was studied using alloxan induced diabetic model in rats. Animals were treated with olive oil at two different doses of 2 mL/kg or 4 mL/kg once daily through oral route and metformin was administered orally at a dose of 150 mg/kg. The serum glucose levels, serum insulin levels and lipid profile were estimated after four weeks of treatment. Olive oil showed antidiabetic effect as indicated by reduction in serum glucose levels and an increase in serum insulin levels. The effect on Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was not evident except in animals treated with higher dose of olive oil (4 mL/kg, p.o) along with metformin (150 mg/kg, p.o). Olive oil also showed favorable effect on lipid profile in diabetic rats. It reduced total cholesterol, triglycerides, VLDL and LDL levels and its effect was potentiated by metformin. The results of the present study further confirm the beneficial properties of olive oil in diabetes and its favorable interaction with metformin, which could help in reducing dose of the later.

Key words: Olive oil, metformin, alloxan, HOMA-IR, insulin, lipid profile.

INTRODUCTION

Olive oil is widely used in many countries with its consumption increasing worldwide due to its use as health supplement. It is assumed that the worldwide consumption of olive oil witnessed a tremendous increase of around 78% between 1990 and 2010 (Bhatnagar *et al.,* 2014). The use of olive oil for maintenance of health in diabetic patients is well-known (Schwingshackl *et al.,* 2017) and it is also reported to reduce incidence of some other chronic diseases such as cardiovascular diseases, obesity and cancer (Covas *et al.,* 2009; Ruiz-Canela and Martinez-Gonzalez, 2011; Perez-Martinez *et al.,* 2011; Esposito *et al.,* 2010; Escrich *et al.,* 2011). Because of these beneficial effects, regular intake of olive oil is believed to reduce mortality (Keys *et al.,* 1986).

Olive oil is known to possess antidiabetic effect in both humans and in rats (Rigacci and Stefani, 2016). We have earlier reported antidiabetic effect of olive oil and augmentation of hypoglycemic effect of metformin in rats (Ahmed *et al*., 2018). Olive oil is also reported to prevent development of type II diabetes in humans (Sebbagh *et al.,* 2009; Ötles *et al.,* 2014).

Consumption of herbs, food and nutraceuticals are known to alter the pharmacological response of several modern drugs (Asher *et al.,* 2017). The interactions are both beneficial and harmful depending on the type of herb and their influence on either pharmacodynamics or pharmacokinetics of modern drugs (Posadzki *et al.,* 2013).

Several nutraceuticals and herbs are used by diabetic patients along with modern medicine to get extra benefit throughout the world. The use of an herb depends on the availability in a particular region. *Gymnema sylvestre*, *Gingko biloba* and *Momordica charantia* (Stage *et al.,* 2015) are some of the examples of herbs used along with oral hypoglycemic drugs to achieve beneficial effect by diabetic patients. These combination of herbs with oral hypoglycemic have been reported to possess additive/ synergistic effect though caution has to be exercised while using them due to steep fall in serum glucose levels and organ toxicity that these herbs may induce in diabetic patients (Shengule *et al.,* 2018). We had earlier reported beneficial effect due to combination of *Momordica charantia* with rosiglitazone in rats (Nivitabishekam *et al.,* 2009) and olive oil with metformin in alloxan induced diabetic rats (Ahmed *et al.,* 2018).

This study was done to determine the effect of olive oil on lipid profile and its effect on insulin release and insulin resistance in alloxan induced diabetic rats. The study also determined the interaction of olive oil with metformin on these parameters.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar rats (20 –250 g) were used. The experimental animals were maintained under standard conditions in an animal house at 25 ± 2º C. The experimental protocol was approved by the scientific committee of the institute.

Chemicals

Olive oil (Aafia Brand, Saudi Arabia) was purchased locally, metformin tablets were used in the study. The serum glucose, serum lipid profile and serum insulin levels were determined using kits available in the local market.

Induction of diabetes

Alloxan (120 mg/kg) was administered intraperitoneally to rats and after 48 h, serum glucose levels were estimated in overnight fasted animals (Debnath *et al.,* 2013). Rats having serum glucose levels of 150 mg/dL or more were considered to have diabetes, and these were assigned to different groups and treated accordingly.

Drug treatment

The animals were divided into different groups as follows;

Group I (normal control) had normal healthy animals and were treated with vehicle (1 mL/kg, p.o).

Group II (diabetic control) – diabetes was induced as mentioned above and the animals received vehicle (1 mL/kg, p.o)

Group III (low dose of olive oil) – consisted of diabetic animals that were treated with olive oil (2 mL/kg, *p.o*) daily.

Group IV (high dose of olive oil) - diabetic animals that received olive oil (4 mL/kg, *p.o*) daily.

Group V (metformin) – diabetic animals that were treated with standard hypoglycemic drug; metformin (150 mg/kg, *p.o*) every day (Mirazi *et al.,* 2015).

Group VI (olive oil low dose + metformin) - animals received combination of metformin (150 mg/kg, *p.o*) + olive oil (2 mL/kg, *p.o*)

Group VII – (olive oil high dose + metformin) – rats treated with combination of metformin (150 mg/kg, *p.o*) + olive oil (4 mL/kg, *p.o*).

All drug treatments were for four weeks and at the end of 4 weeks, rats were fasted overnight, and the retro-orbital plexus was punctured under anesthesia to draw blood. Serum was used for estimation of total cholesterol, triglycerides, glucose levels, HDL, LDL using kits while the VLDL was calculated using standard formula (Wilson *et al.,* 1985). Serum insulin levels were determined using rat insulin kit (Elabscience, USA). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula (Salgado *et al.,* 2010)

$$\text{HOMA-IR} = \frac{\text{Fasting insulin (microU/L) x fasting glucose (nmol/L)}}{22.5}$$

Statistical analysis

Results are given as mean ± SEM. The statistically significant difference was determined using one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test.

RESULTS

Effect on serum glucose levels, insulin and HOMA-IR:

Administration of alloxan produced diabetes in all animals. It was confirmed by a significant increase in serum glucose levels ($p<0.05$) compared to control animals. Olive oil at both doses reduced the blood glucose level in a dose dependent manner when compared to diabetic control animals that were injected with alloxan and received only vehicle ($p<0.05$). As expected, metformin showed good antidiabetic effect. The combination of olive oil with metformin showed significantly more reduction in serum glucose when compared to animals that receive olive oil alone ($p<0.05$). Determination of insulin in the serum revealed that olive oil at both doses increase insulin release from pancreas of alloxan treated animals. A similar effect was observed with metformin. However, there was no significant difference between olive oil treated animals and animals that received olive oil along with metformin, ruling out any additive or synergistic effect on insulin release. HOMA-IR, an indicator of insulin resistance revealed that in diabetic control animals, the insulin resistance was lower than normal control animals. There was no

significant effect on HOMA-IR between diabetic control animals and groups of animals that received different treatments except in animals treated with high dose of olive oil (4 mL/kg, *p.o*) along with metformin (Table 1).

Table 1. Effects on serum glucose levels, insulin and HOMA-IR.

Groups	Serum Glucose (mg/dL)	Insulin (IU/mL)	HOMA-IR
Control (Vehicle 1 mg/kg, *p.o*)	106.06 ± 3.967 f	11.96 ± 0.202 a	2.69±0.035
Diabetic control (Vehicle 1 mg/kg, *p.o*)	397.40 ± 7.316 a	1.78 ± 0.059 d	1.37±0.065
Olive oil (2 mL/kg, *p.o*)	287.66 ± 1.642 b	2.13 ± 0.021 c	1.33 ± .0102
Olive oil (4 mL/kg, *p.o*)	241.06 ± 1.642 cd	2.54 ± 0.048 ab	1.41 ± 0.028
Metformin (150 mg/kg, *p.o)*	251.66 ± 1.319 c	2.35 ± 0.045 bc	1.45 ± 0.030
Olive oil (2 mL/kg, *p.o*) + metformin (150 mg/kg, *p.o)*	230.73 ± 1.354 d	2.50 ± 0.021 b	1.33 ± 0.015
Olive oil (4 mL/kg, *p.o*) + metformin (150 mg/kg, *p.o)*	213.73 ± 0.917 e	2.50 ± 0.041 b	1.21 ± 0.022
$LSD_{0.05}$	13.094	0.284	

All values are mean ± SEM, n=15, Similar letters are non-significant in each column according to Duncan Multiple range test at $p<0.05$.

Effect on lipid profile:

Olive oil alone or in combination with metformin produced a reduction in total cholesterol, triglycerides, VLDL and LDL while a significant increase in HDL levels was observed. Metformin did not produce any significant decrease in total cholesterol, but it had favorable effect on other lipid parameters (Table 2)

Table 2. Effects on lipid profile.

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control (Vehicle 1 mg/kg, *p.o*)	106.66 ± 1.059 e	99.13 ±1.330 e	19.82 ± 0.266 e	43.40 ± 0.659 f	43.82 ± 0.645 d
Diabetic control (Vehicle 1 mg/kg, *p.o*)	252.66 ± 1.095 a	246.60 ± 1.301 a	51.37 ± 0.257 a	210.90 ± 1.435 a	30.76 ± 0.650 f
Olive oil (2 mL/kg, *p.o*)	218.86 ± 0.928 b	167.46 ± 1.424 c	33.16 ± 0.209 c	143.54 ± 9.442 c	40.82 ± 0.337 e
Olive oil (4 mL/kg, *p.o*)	199.73 ± 0.765 c	136.80 ± 0.752 d	27.36 ± 0.150 d	118.81 ± 0.748 d	53.56 ± 0.522 b
metformin (150 mg/kg, *p.o)*	249.40 ± 6.560 a	188.13 ± 1.902 b	37.61 ± 0.380 b	168.12 ± 1.711 b	41.56 ± 0.304 de
Olive oil (2 mL/kg, *p.o*) + metformin (150 mg/kg, *p.o)*	207.20 ± 0.788 c	180.00 ± 0.972 b	35.93 ± 0.197 b	124.80 ± 0.807 d	45.76 ± 0.673 c
Olive oil (4 mL/kg, *p.o*) + metformin (150 mg/kg, *p.o)*	186.73 ± 1.392 d	145.17 ± 0.897 d	29.09 ± 0.178 d	100.18 ± 0.836 e	57.76 ± 0.511 a
$LSD_{0.05}$	11.352	12.49	2.639	10.349	2.258

All values are mean ± SEM, n=15, Similar letters are non-significant in each column according to Duncan Multiple range test at $p<0.05$.

DISCUSSION

The results of present study showed that olive oil increased secretion of insulin only at higher doses while it did not significantly affect the insulin releasing effect of metformin at both the tested doses. With respect to HOMA-IR, combination of higher dose of olive oil (4 mL/kg, *p.o*) with metformin significantly reduced the HOMA-IR when compared to diabetic control rats.

There are several methods for induction of diabetes in rats. Pancreatic toxicants such as streptozotocin and alloxan are commonly used (Vogel, 2002). In our study, we used alloxan to induce diabetes. It damages β-cells of

islets of Langerhans and hence it is believed to induce type-I diabetes. Oral hypoglycemics are known to reduce hyperglycemia induced by alloxan indicating that it does not completely damage the pancreas and few pancreatic cells will still be viable after alloxan induced pancreatic damage (Agwaya *et al.,* 2016; Das *et al.,* 2016; Tang *et al.,* 2015).

In the present study, metformin, olive oil and their combination reduced serum glucose levels and increased insulin secretion confirming that alloxan does not completely damage the pancreas and olive oil and metformin reduce serum glucose levels by increasing insulin secretion.

The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was also calculated to determine the effect on insulin resistance. An increased HOMA-IR is an indicator of insulin resistance while a lower HOMA-IR signifies sensitivity to insulin. The results of the present study suggest that induction of diabetes by alloxan increases HOMA-IR. This is in contrast to many earlier reports that induction of diabetes using alloxan/streptozotocin increases HOMA-IR (Aissaoui *et al.,* 2017; Tuorkey, 2016). We assume that decreased secretion of insulin after pancreatic damage by alloxan led to an increase in insulin sensitivity. The HOMA-IR may increase in other models of diabetes such as those induced by using high fat diet and it may not increase after chemically induced diabetes. None of the treatments affected HOMA-IR significantly except combination of high dose of olive oil and metformin wherein a decrease in HOMA-IR was observed. This result is clinically relevant as it indicates that even at very low serum insulin levels, combination of olive oil and metformin may still sensitize the tissues to the insulin action and may help in reducing serum glucose levels. Our results are supported by an earlier study that olive oil increases glucose utilization in muscles through its chemical constituent - oleuropein (Fujiwara *et al.,* 2017). Metformin is known to increase utilization of glucose by muscles and decrease glucose absorption from intestine (Goodman *et al.,* 2011).

Influence of olive oil and metformin on lipid profile in diabetic rats show that olive oil possesses good hypolipidemic effect and this effect is increased when olive oil is administered along with metformin. There is plethora of information about use of olive oil to lower blood sugar levels in diabetic patients and in prevention of cardiovascular complications (Covas *et al.,* 2009; Ruiz-Canela and Martinez-Gonzalez, 2011; Perez-Martinez *et al.,* 2011; Esposito *et al.,* 2010; Escrich *et al.,* 2011). Metformin is also reported to possess mild hypolipidemic effect and our study further confirmed its hypolipidemic effect in diabetic animals. The effect was observed on all the studied parameters that included triglycerides, total cholesterol, LDL, VLDL and HDL.

The benefits of herb-drug interactions is well known and several herbs and food additives have been reported to enhance therapeutic benefits when combined with modern drugs. Of late, herb-drug interactions is being recognized as a cause of potential toxicity and many organizations recommend monitoring the use of herbs that are used for the management and treatment of diseases (Posadzki *et al.,* 2013; Stage *et al.,* 2015).

There are several reports on the beneficial effect of many herbs when used along with antidiabetic agents (Shengule *et al.,* 2018). However, very less information is available on the influence of olive oil on antidiabetic medications. As mentioned earlier, we had earlier reported a favorable interaction of olive oil with metformin on alloxan induced diabetic drugs (Ahmed *et al.,* 2018). This study further confirms favorable interaction between olive oil and metformin by studying effect on serum insulin levels and HOMA-IR.

Hyperlipidemia and its associated complication are very common among diabetic patients. Hence, a variety of hypolipidemic agents such as statins, fibrates etc., are prescribed to diabetic patients along with antidiabetic drugs. The hypolipidemic agents are known to possess several adverse effects including hyperglycemia that is observed with statins (Jain *et al.,* 2017; Fathallah *et al.,* 2015. The results of the present study suggest that hypolipidemic drugs and food products or food additives may act as suitable replacement for modern hypolipidemic drugs. Use of herbs and food products may significantly lower the risk of adverse drug reactions among diabetic patients. As mentioned earlier, in Mediterranean countries, olive oil is widely consumed by patients suffering from cardiovascular complications and diabetes (Buckland *et al.,* 2015). There are several studies that report that Mediterranean diet helps to prevent diabetes and cardiovascular complications (Esposito *et al.,* 2015; Esposito and Giugliano, 2014). Hence, it can be suggested that consumption of olive oil may at least in part contribute to the less prevalence of diabetes and hyperlipidemia in the Mediterranean countries. Furthermore, consumption of olive oil by diabetic patients may help to achieve better glycemic control and prevent diabetic associated complications such as hyperlipidemia and cardiovascular problems.

Though a beneficial effect was observed with combination of olive oil with metformin, we would like to stress that further studies should be carried out to determine if co-administration of olive oil with metformin can increase the risk of adverse effects of metformin or induce new unknown adverse drug reactions. Furthermore, studies on pharmacokinetic interactions between olive oil and metformin may provide more information about this interaction that may help to predict the beneficial or harmful effect of this combination.

Conclusion

Olive oil augmented the hypoglycemic action of metformin through an increase in insulin release and by decreasing HOMA-IR. Olive oil and its combination with metformin produces beneficial effect on lipid profile in alloxan induced diabetic rats.

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