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EVALUATION OF EXTRA VIRGIN OLIVE OIL "EVOO" ON THE PHARMACODYNAMICS OF GLIBENCLAMIDE IN DIABETIC RATS

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ABSTRACT

Background: Diabetes growing steadily all over the world irrespective of health care efforts to prevent it. Many anti-diabetics were present and new generations continue to be introduced into the market. Glibenclamide is an antidiabetic, which exerts its therapeutic effect by triggering insulin secretion. The olive tree has been recognized for a long time as a source of bioactive polyphenols which have an anti-hyperglycemic effect on diabetic rats.

Aim of the work: To study the effect of extra virgin olive oil (EVOO) on the pharmacodynamics of glibenclamide in diabetic rats.

Materials and methods: This study was carried on 50 Male adult albino rats (200-250g). They were divided into 2 groups. **Group 1:** included "10 rats" served as "Control group" received single i.v. injection of citrate buffer, in a volume equal to that used as a solvent for streptozotocin (STZ) used to induce diabetes in test groups. **Group 2**: included diabetic rats"40 rats" which were injected by single intraperitoneal injection of a freshly prepared solution of STZ (dissolved in cold 0.01 M citrate buffer, pH 4.5) in a dose of 55 mg/kg body weight. The diabetic rats were divided into 4 **subgroups. Group A:** Diabetic rats concurrently treated with saline. **Group B:** Rats of this group were treated orally by 0.3 mg/kg of glibenclamide daily for 6 weeks. **Group C:** Rats of this group were treated orally by (1mL/100gbw) of Extra Virgin Olive Oil (EVOO) daily for 6 weeks. **Group D:** Rats of this group

were treated orally by (0.3 mg/kg) of glibenclamide and (1mL/100g.bw) of Extra Virgin Olive Oil (EVOO) daily for 6 weeks. Then blood samples were collected and examined for serum glucose, lipid profile, liver and kidney functions. Rats were sacrificed; livers were obtained and prepared for histological examination.

Results: There was statistically significant increase of glucose, triglycerides, low density lipoprotein, total cholesterol, alanine transaminase, aspartate transaminase, urea and creatinine, and significant decrease of high density lipoprotein in the diabetic group when compared to control group. Administration of either glibenclamide or EVOO or combination together was associated with improvement of biochemical alterations associated with diabetes with superiority of glibenclamide. Histopathological, normal control revealed normal structure of the liver and kidney, while in diabetic group, the liver tissues showed loss of normal lobular architecture, with liver cell apoptosis (the cells are shrunken, the nuclei were dark stained, fragmented or faint). Each of glibenclamide, EVOO was able to prevent histopathological abnormalities in both liver and kidney.

Conclusion: The combination of EVOO with glibenclamide represents a valuable combination to improve diabetes and its complications.

Keywords: Antidiabetic; Glibenclamide; Extra-virgin olive oil; Diabetes mellitus

INTRODUCTION

Glibenclamide is an antidiabetic, which exerts its therapeutic effect by triggering insulin secretion **[1]**. It also promotes islet cell expression and function of connexin 36 (Cx36), **[2]**, a gap junction protein which markedly shares to control secretion and survival of beta cells **[3].** Previous studies have proposed that sulphonylureas may mitigate the hyperglycemia which develops with age in the non-obese diabetic mice (NOD), a widely used model of type I diabetes. However, these studies have also provided conflicting evidence about such a protective role **[4]**. The use of the olive tree fruit and oil in human nutrition, health and cosmetics was documented from about 6000 years ago and continues to have its role **[5]**. The use of olive fruit in health and medicine had been widely known since these ancient times. However, epidemiological studies assessing the use of olive only was recently introduced and revealed positive effects on human health with regular consumption of extra virgin olive oil (EVOO). EVOO work as an antioxidant, reduce incidence of cancer and improve cardiovascular health **[6-8]**. The olive tree has been recognized for a long time as a source of bioactive polyphenols, such as oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosol **[9]**. Olive polyphenols had an anti-hyperglycemic effect on diabetic rats **[10]**. Although the mechanism by which they attenuate hyperglycemia is still not well known. However, particular attention has been paid

to hydroxytyrosol **[11]**. Since olive oil has been recommended in the literature **[12]** as a remedy for the treatment of diabetes, the present work was designed to study the effect of extra virgin olive oil (EVOO) on the pharmacodynamics of glibenclamide in diabetic rats.

MATERIAL AND METHODS

Drugs and chemicals:

- Streptozotocin (STZ) (Sigma chemical company).
- Citrate buffer Ph 4.5 (citric acid+ sodium citrate) in distilled water 0.1 moles.- Extra Virgin Olive Oil is obtained from the market produced by Sinawy Co.
- Glibenclamide was obtained from a local pharmacy and was dissolved in distilled water containing 0.9% (wt/vol) sodium chloride for oral administration.

Kits:

- Kits for measurement of lipid profile were obtained from (Diamond Diagnostic Company, Egypt).
- Kits for measurement of serum glucose were obtained from (Biodiagnostic, Giza, Egypt).
- Kits for measurement of liver enzymes (ALT & AST), were obtained from (Diamond Diagnostic Company, Egypt).
- Kits for measurement of kidney functions were obtained from (Diamond Diagnostic Company, Egypt)

Animals:

Experiment were conducted using 50 male adult albino rats weighing (200-250g), bred in the animal house of pharmacology department of AL-Azhar University. The animals were handled according to the guide lines of local ethical committee which comply with the international laws for use and care of laboratory animals. The animals were allowed to acclimatize for 2 weeks before the experiment. The animals were housed four per cage under controlled temperature (23±1°C) in polypropylene cages inside a well-ventilated room, relative humidity 40% and 12 hour light/dark cycle. They were fed a standard commercial pellet diet and water ad libitum. The diet consists of 71% carbohydrate, 18% protein, 7% fat, 4% salt mixture and adequate minerals and vitamins.

Induction of Diabetes Mellitus:

Streptozotocin was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 min. Diabetes was induced in rats by single intraperitoneal injection of a freshly prepared solution of streptozotocin in a dose of 55 mg/kg body weight **[13]**. After STZ injection, ratswere received 10%

(wt/vol) of glucose in the drinking water for 3 days to compensate for hypoglycemia which results from insulin release from beta cells of the islets of Langerhans due to their destruction by streptozotocin. The blood glucose concentration was measured 4 days from the day of STZ injection. The rats with blood glucose higher than 250mg/dl were considered diabetics and were used in the experiment.

Experimental design: They were divided into 2 groups:

Group 1: Included "10 rats" served as "Control group" received single i.v. injection of citrate buffer, in a volume equal to that used as a solvent for STZ used to induce diabetes in test groups.

Group 2: Included diabetic rats "40 rats" which were injected by single intraperitoneal injection of a freshly prepared solution of streptozotocin in a dose of 55 mg/kg body weight. The diabetic rats were divided into 4 subgroups.

Group A: Rats of this group were treated orally by saline for 6 weeks.

Group B: Rats of this group were treated orally by 0.3 mg/kg of glibenclamide daily for 6 weeks.

Group C: Rats of this group were treated orally by (1mL/100gbw) of Extra Virgin Olive Oil (EVOO) daily for 6 weeks.

Group D: Rats of this group were treated orally by (0.3 mg/kg) of glibenclamide and (1mL/100g.bw) of Extra Virgin Olive Oil (EVOO) daily for 6 weeks.

Collection of blood samples and biochemical analysis:

At the end of the study (6 weeks), blood samples were collected from the retro-orbital venous plexus of rat eye by using heparinized capillary tubes. The collected blood samples were then centrifuged (Cooling centrifuge, sigma 2 k 15) at 300 round/minute for 30 minutes. Then the serum was transferred into clean vials and stored at -18°C for biochemical parameters determination **[14]**. Rats were sacrificed; livers were obtained and prepared for histological examination.

Statistical analysis:

All values are expressed as mean ± SEM. Data were statistically analyzed using independent samples student (t) test for comparison between two groups. Significance was set at $p \leq 0.05$. Data were computed for statistical analysis by using statistical package for social science (SPSS), version 16 (SPSS Inc, US), running on IBM compatible computer.

RESULTS

In the present study, glucose levels, triglycerides, LDL, total cholesterol, ALT, AST, urea, serum creatinine and 24-hour urinary albumin were significantly

increased while HDL was significantly decreased in diabetic when compared to control group **(Table 1), (Figure 1)**. When comparing diabetic rats treated with glibenclamide to diabetic non treated group (A), there was significant decrease of glucose, triglycerides, LDL, total cholesterol, ALT, AST, urea and 24-hour urinary albumin. However, no significant difference regarding HDL was encountered. In addition, values of glucose, TG, total cholesterol, ALT, AST, urea, creatinine and 24-hour urinary albumin were significantly decreased among diabetic plus EVOO when compared to diabetic non treated group (A). However, no significant difference was found regarding HDL and LDL. Finally, there was significant decrease of glucose, TG, LDL, total cholesterol, ALT, AST, urea, creatinine and 24 hours urinary albumin while there was significant increase of HDL in diabetic with glibenclamide plus EVOO when compared to diabetic group (A) **(Table 2), (Figure 2)**.

When the effect of glibenclamide compared to EVOO, there was no significant difference between both groups as regard to glucose, triglycerides, HDL and creatinine. However, glibenclamide was significantly associated with marked decrease of LDL, total cholesterol, ALT, AST, urea and 24 hours urinary albumin when compared to EVOO. Furthermore, the combination of glibenclamide plus EVOO was associated with significant decrease of LDL, ALT, AST, Urea, creatinine and 24 hours urinary albumin when compared to glibenclamide group. In addition, the combination of glibenclamide and EVOO was associated with significant decrease of LDL, total cholesterol, ALT, AST, urea, creatinine and 24 hours urinary albumin with significant increase of HDL when compared to EVOO alone **(Table 3)**.

Histopathology: In normal (negative) control group, the liver appears normal in structure, with normal hepatic lobule, where cells were arranged radially around the central vein (CV). The cytoplasm was acidophilic, the nuclei is rounded, vesicular and euchromatic with nucleoli. Groups of hepatocytes were intervened by thin-walled blood sinusoids, with Kupffer cells lining the sinusoids **(Picture 1)**. In diabetic group, the liver tissues showed loss of normal lobular architecture, with liver cell apoptosis (the cells are shrunken, the nuclei were dark stained, fragmented or faint) **(Picture 2)**. Histological sections of the glibenclamide, EVOO and both drugs showed more or less normal structure of the liver, with no apparent pathological alterations, as shown in negative control group.

Table 1: Comparison between control and diabetic groups regarding biochemical results

	Control group		Diabetic group		P
	Mean	S.D	Mean	S.D	
Glucose	100.30	8.94	329.30	37.12	<0.001*
TG	180.20	15.10	256.70	16.11	<0.001*
HDL	29.40	4.62	19.70	3.33	<0.001*
LDL	28.80	4.18	75.30	13.27	<0.001*
Total cholesterol	93.20	6.71	201.60	9.52	<0.001*
ALT	21.60	1.35	60.60	6.02	<0.001*
AST	19.20	1.32	51.00	9.63	<0.001*
Urea	21.90	4.68	71.90	6.61	<0.001*
Creatinine	0.62	0.13	2.03	0.37	<0.001*
Urinary albumin (g/24h)	0.57	0.20	3.15	0.76	<0.001*

Table 2: Comparison between diabetic and treatment groups regarding biochemical results

	Diabetic group		Diabetic + glibenclamide		Diabetics +EVOO		Diabetics+ glibenclamide + EVOO		P1	P2	P3
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D			
Glucose	329.30	37.12	103.50	5.42	105.90	3.41	101.50	7.20	<0.001*	<0.001*	<0.001*
TG	256.70	16.11	182.60	14.71	182.90	14.34	183.50	10.37	<0.001*	<0.001*	<0.001*
HDL	19.70	3.33	26.20	11.03	21.40	3.03	28.30	3.71	0.091	0.248	<0.001*
LDL	75.30	13.27	51.40	8.36	70.20	13.01	32.40	4.84	<0.001*	0.397	<0.001*
Total cholesterol	201.60	9.52	93.90	5.84	108.30	6.02	94.40	6.92	<0.001*	<0.001*	<0.001*
ALT	60.60	6.02	32.10	2.02	36.60	3.31	27.40	2.22	<0.001*	<0.001*	<0.001*
AST	51.00	9.63	29.10	1.85	32.80	3.97	24.80	2.53	<0.001*	<0.001*	<0.001*
Urea	71.90	6.61	28.10	5.17	32.90	3.60	23.70	4.24	<0.001*	<0.001*	<0.001*
Creatinine	2.03	0.37	1.01	0.15	0.99	0.23	0.69	0.11	<0.001*	<0.001*	<0.001*
Urinary albumin (g/24h)	3.15	0.76	1.42	0.30	2.06	0.33	1.05	0.22	<0.001*	0.001*	<0.001*

p1 = comparison between diabetic groups and diabetic plus glibenclamide group; p2= comparison between diabetic group and diabetic plus EVOO group; p3= comparison between diabetic group and diabetic plus glibenclamide and EVOO group; * = significant

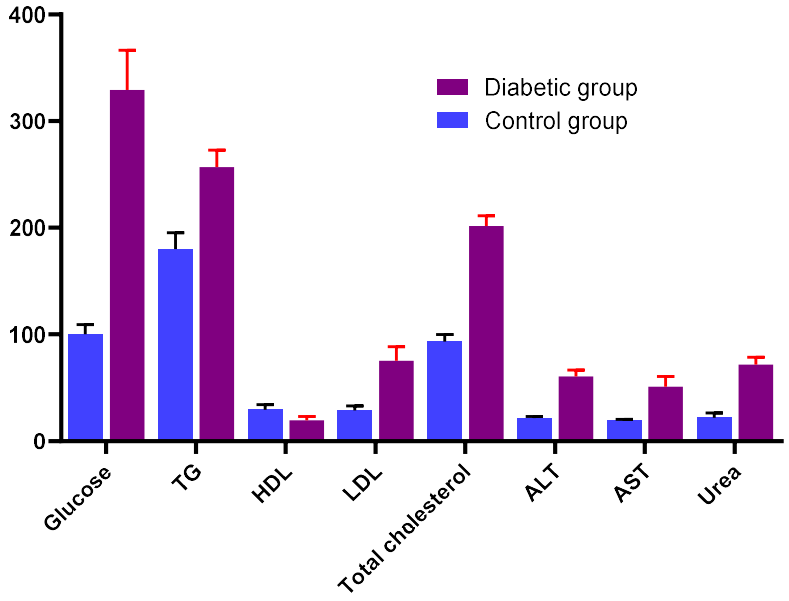


Figure 1: Comparison between Biochemical results of control and diabetic group

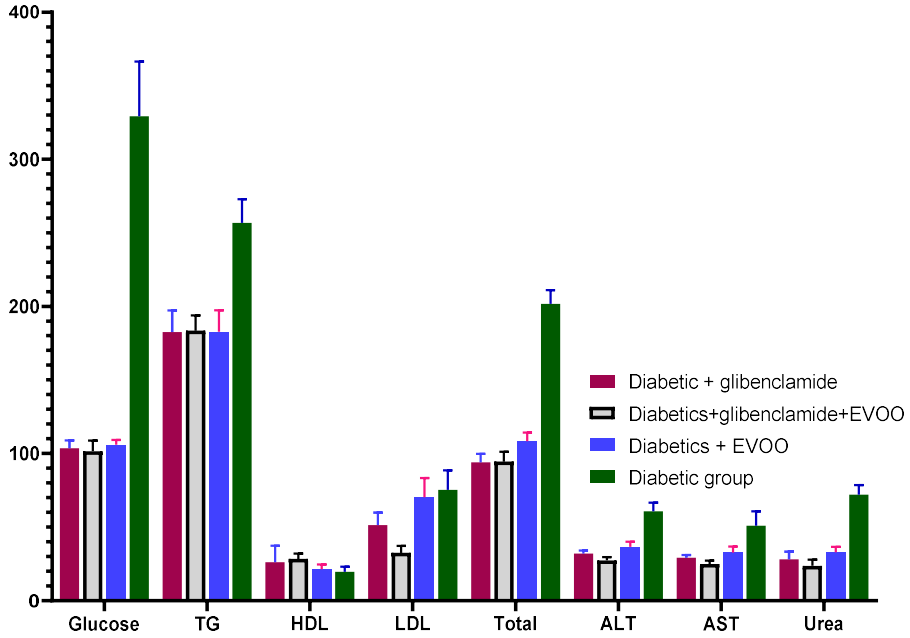
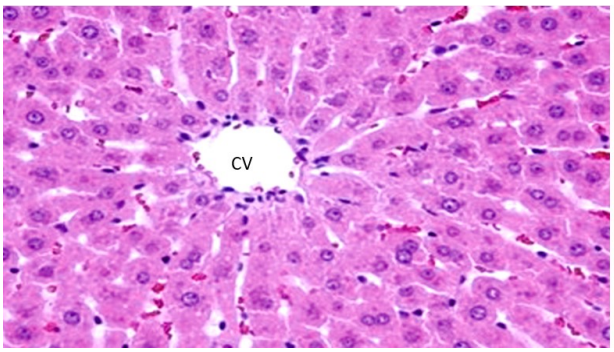


Figure 2: Comparison between diabetic and treatment groups regarding biochemical results

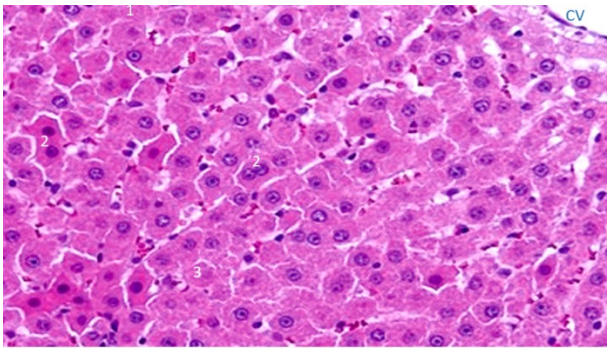
Table 3: Comparison between treatment groups regarding biochemical results

	Diabetic +glibenclamide		Diabetics +EVOO		Diabetics+ glibenclamide + EVOO		P1	P2	P3
	Mean	S.D	Mean	S.D	Mean	S.D			
Glucose	103.50	5.42	105.90	3.41	101.50	7.20	0.252	0.492	0.098
TG	182.60	14.71	182.90	14.34	183.50	10.37	0.964	0.876	0.916
HDL	26.20	11.03	21.40	3.03	28.30	3.71	0.201	0.575	<0.001*
LDL	51.40	8.36	70.20	13.01	32.40	4.84	0.001*	<0.001*	<0.001*
Total cholesterol	93.90	5.84	108.30	6.02	94.40	6.92	<0.001*	0.863	<0.001*
ALT	32.10	2.02	36.60	3.31	27.40	2.22	0.002*	<0.001*	<0.001*
AST	29.10	1.85	32.80	3.97	24.80	2.53	0.016*	<0.001*	<0.001*
Urea	28.10	5.17	32.90	3.60	23.70	4.24	0.027*	0.05*	<0.001*
Creatinine	1.01	0.15	0.99	0.23	0.69	0.11	0.823	<0.001*	0.002*
Urinary albumin (g/24h)	1.42	0.30	2.06	0.33	1.05	0.22	<0.001*	0.006*	<0.001*

P1= comparison between glibenclamide and EVOO groups; P2= Comparison between glibenclamide and Glibenclamide plus EVOO groups; P3= Comparison between EVOO and Glibenclamide plus EVOO groups



picture(1): Photmicrograph of the liver in negative control group, showing normal hepatic lobule, where hepatic cells arranged in a radial manner around central vein (CV), and kupffer cells lining the sinusidal spaces (H & E x 200).



picture (2): Loss of normal architecture with numerous apoptotic dark-stained shrunken hepatocytes with (absent nuclei [1], bi-nucleated cells [2] or small degenerated nuclei [3].(H & E x 300).

DISCUSSION

In the present work, the potential protective effects of anti-diabetic glibenclamide and Extra Virgin Olive OIL (EVOO) on both laboratory findings of blood sugar, lipid profile, liver and kidney functions was investigated, as well as , the histopathology of the liver. Results revealed that, both glibenclamide and EVOO provided protective effects, as both normalized blood sugar and triglycerides. Glibenclamide additionally normalizes HDL. When both drugs used simultaneously, all laboratory tests were nearly normalized except ALT and AST. Histopathological examination revealed harmful effects on liver tissues, and each of both drugs was able to prevent development of pathological effects of liver tissue. Glibenclamide found to be superior in prevention of alterations in LDL, total cholesterol, ALT, AST and urea.

In accordance with results of the present work, **[15]** who reported that, there was an abnormality in lipid profile, which is a common complication of diabetes mellitus. In addition, our work agrees **[16]** who reported that, there was a significant increase in triglycerides in diabetic rats when compared to normal control group. Also, **[15]** reported significant abnormalities in all lipid profile components in diabetic rats when compared to control group.

Results of the present work supported b the results obtained by **[17, 18]** who reported that, STZ-induced diabetic mice showed an increase in ALT and AST, when compared to control group.

On the other side, **[19]** could not find significant difference between normal control and diabetic rats regarding lipid profile, with non significant difference found between treated groups, negative control or diabetic groups. They attributed this non-significant difference to short period of their study which was not sufficient to develop biochemical alterations. Also, **[20]** reported no significant alterations of lipids were observed in diabetic rats.

Our results are consistent with **[21]** who reported that, there was a significant increase in serum concentrations of urea and creatinine in diabetic group when compared to control group. **[22]** could not observe any significant increase in blood urea or creatinine in diabetic when compared to control group, while **[19]** found significant increase of urea but not creatinine, and proposed that, this increase in blood urea could be attributed to other factors other than kidney dysfunction (e.g., dietary factors), irrespective of they reported significant histopathological changes of liver, kidney and pancreas of diabetic rats when compared to normal control rats; the findings which contradict

the biochemical findings. Anyway, results of the present work are consistent with **[23]** who reported that, liver of diabetic rats showed significant histopathological abnormalities (degenerative changes; loss of lobular structure and apoptosis).

The protective effects of EVOO come in agreement with **[24]** who reported a protective effect (prevention of histopathological changes among liver tissues) with administration of olive oil. They added, pretreatment by olive oil led to preservation the normal structure of the liver indicating a prophylactic role.

The protective role of EVOO is attributed to its phenolic compounds which associated with increased HDL levels and improvement of endothelial function **[25]**. In addition, such polyphenols could alter glucose metabolism via inhibition of carbohydrate digestion, carbohydrate absorption, decreased glucose release from the liver, or stimulation of glucose uptake in peripheral tissues **[26].** Another mechanism was thought to be exerted through antioxidant properties of EVOO. It could decrease production of advanced glycosylated end production (e.g., Hemoglobin A1c) **[27].** The administration of such polyphenols was reported to be associated with enhanced insulin secretion and sensitivity, with decreased serum glucose levels and HA1c **[28, 29]**.

Furthermore, **[30]** in his systematic review and meta-analysis claimed that, olive oil had a favorable effects on type-2 diabetes risk and variables of glycemic control. They added, extra-virgin olive oil as an integral part of a Mediterranean diet, represents a suitable constituent of a balanced diet.

In summary, the biochemical (mainly) and histopathological data from this study revealed that, the combination of EVOO with oral hypoglycemic glibenclamide represent a valuable combination to prevent hyperglycemia and its complications.

Significance of the study:

This study provided evidence on the efficacy of EVOO to protect against diabetes and its glucose lowering effects. In addition, results of the study confirmed the role of glibenclamide (an old anti-diabetic drug); and compared the effects of glibenclamide with EVOO for the first time. We must stress that, at the present time, EVOO alone could not be used as antidiabetic drug (although serum glucose levels were controlled among studied animals), but as an adjuvant with antidiabetic. Further clinical evidence is required to search the anti-diabetic mechanisms of EVOO.

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