

SUPPLEMENTARY MATERIAL

Effects of Emulsion Formulations of Oleuropein Isolated from Ethanol Extract of Olive Leaf in Diabetic Rats

Determination of the Ideal Doses

A preliminary study was carried out to determine the oral administration doses of oleuropein (OL). In the preliminary study, the rats were divided into eight groups, with each group consisting of 7 rats. The groups were characterized as follows:

Group C consisted of untreated rats,

Group CD consisted of diabetic rats

Group DM20 consisted of normalrats that received OL at a dose of 20 mg/kg b.wt./day,

Group DM40 consisted of diabetic rats that received OL at a dose of 40 mg/kg b.wt./day,

Group DM60 consisted of diabetic rats that received OL at a dose of 60 mg/kg b.wt./day,

Group DM100 consisted of diabetic rats that received OL at a dose of 100 mg/kg b.wt./day,

Group DM150 consisted of diabetic rats that received OL at a dose of 150 mg/kg b.wt./day.

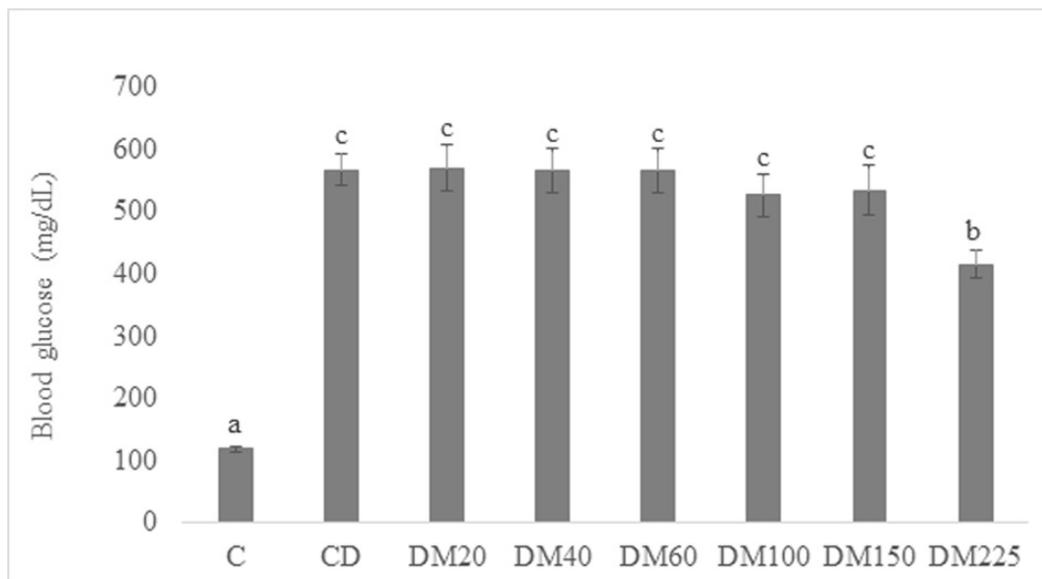
Group DM225 consisted of diabetic rats that received OL at a dose of 225 mg/kg b.wt./day.

Solutions were first prepared by suspending OL (at six different doses of 20, 40, 60, 100, 150 and 225 mg/kg b.wt., respectively) in distilled water before starting the experiment. The STZ-induced diabetic rats were then treated with the administrations of the solutions by oral gavage (once daily for 30 days). At the end of 30 days, visible signs of lethality were seen in the diabetic rats exposed to OL at the doses of 20, 40, 60 and 100 mg/kg b.wt. Also, the rats showed body weight loss (data not shown), and the animals had withered and yellow hair throughout the preliminary study, but there were no such effects when the rats were exposed to OL at the doses of 150 and 225 mg/kg b.wt. Furthermore, both water consumption and urine volume increased in the rats in the CD, DM20, DM40, DM60, DM100 and DM150 groups when compared with those of the rats in the group DM225.

The blood glucose levels of the diabetic rats in the CD, DM20, DM60, DM40, DM100 and DM150 groups were significantly higher when compared with those of the rats in the DM225 group ($p < 0.05$). The variations in the blood glucose between the groups for 30 days were shown in Figure S1. On the other hand, at the end of the 30-day experiment, it was observed that the serum AST and ALT levels were significantly elevated in the CD, DM20, DM40, DM60 and DM100 groups when compared with those of the rats in the group C. On the contrary, a significant decrease in serum AST and ALT levels of the rats in the DM150 and DM225 groups was observed ($p < 0.05$). Serum AST and ALT levels of all the groups were shown in Table S1.

In light of these findings, in the current study, for the oral administrations of OL, the doses of 150 and 225 mg/kg b.wt. were considered to be the ideal doses.

Figure S1. Blood glucose levels of the groups at the end of 30 days.



Values are expressed as mean ± SD (n=5). Bars with different letters differ significantly; p<0.05. C: normal control; CD: diabetic control; DM20: diabetic + OL at a dose of 20 mg/kg b.wt.; DM40: diabetic + OL at a dose of 40 mg/kg b.wt.; DM60: diabetic + OL at a dose of 60 mg/kg b.wt.; DM100: diabetic + OL at a dose of 100 mg/kg b.wt.; DM150: diabetic + OL at a dose of 150 mg/kg b.wt.; DM225: diabetic + OL at a dose of 225 mg/kg b.wt.

Table S1. Serum AST and ALT levels of the groups.

Groups	AST (U/L)	ALT (U/L)
C	153.94± 18.58 ^a	55.05± 5.97 ^a
CD	908.81± 94.28 ^d	424.37± 46.10 ^c
DM20	821.80± 100.34 ^d	414.01± 43.24 ^c
DM40	902.18± 85.48 ^d	463.80± 48.47 ^c
DM60	886.54± 89.90 ^d	448.98± 45.97 ^c
DM100	905.70± 90.44 ^d	465.43± 40.98 ^c
DM150	508.84± 52.10 ^c	195.96± 15.91 ^b
DM225	309.20± 32.32 ^b	154.26± 17.69 ^b

Values are expressed as mean ± SD (n=5). The groups in the same column with different superscript letters are statistically significant (p<0.05). C: normal control; CD: diabetic control; DM20: diabetic + OL at a dose of 20 mg/kg b.wt.; DM40: diabetic + OL at a dose of 40 mg/kg b.wt.; DM60: diabetic + OL at a dose of 60 mg/kg b.wt.; DM100: diabetic + OL at a dose of 100 mg/kg b.wt.; DM150: diabetic + OL at a dose of 150 mg/kg b.wt.; DM225: diabetic + OL at a dose of 225 mg/kg b.wt.