

Toxicity of Olive Leaves (*Olea europaea* L.) in Wistar Albino Rats

Sawsan A. Omer, M.A. Elobeid, M.H. Elamin, Z.K. Hassan, P. Virk, M.H. Daghestani, E.M. Al-Olayan, Nadia A. Al-Eisa and Z.M. Almarhoon

Department of Zoology, College of Science, King Saud University, University Centre for Women Students, P.O. Box 22452, Riyadh 11495, Saudi Arabia

Corresponding Author: Sawsan A. Omer, Department of Zoology, College of Science, King Saud University, University Centre for Women Students, P.O. Box 22452, Riyadh 11495, Saudi Arabia Tel: 00966504140713 Fax: 0096614678514

ABSTRACT

The aim of this study was to evaluate the effect of Olive Leaf Extracts (OLE) on the haematology and biochemistry as well as on the liver and kidney of rats fed on the extracts for 6 weeks. Thirty Wistar albino rats were divided into five groups, Group 1 fed regular diet without OLE (control group), group 2 (fed 0.2% ole), group 3 (fed 0.4% ole), group 4 (fed 0.7% OLE) and group 5 (fed 0.9% OLE) for 6 weeks. Serum concentrations of Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Total Bilirubin (TBil) and cholesterol, glucose, triglycerides as well as hematological profiles were determined in the present study for each group of rats. There was a significant increase in the serum levels of ALP and total bilirubin in groups 3 and 4 and 5 compared to the control group. There was also significant decrease in the serum triglyceride, glucose and cholesterol in test groups as compared to the control group. The haematological profile showed significant decrease in the values of red blood cell counts, haemoglobin and packed cell volume of the animals in group 5. Microscopically both liver and kidneys showed histological alterations in the form of fatty cytoplasmic vacuolation, necrosis of the hepatocytes and a slight hemorrhage was recorded in the kidneys of the experimental animals especially those fed 0.9% olive leaf extract. The olive leaves extract should be handled with care in arts and other animals and special attention should be paid when using OLE for longer periods of time and at higher doses as it may result in an undesirable effect on liver and kidneys as it has been shown in the present study.

Key words: Haematology, serum chemistry, histopathology, *Olea europaea*, rats, hepatorenal effects

INTRODUCTION

The olive tree (*Olea europaea*) is native to the Mediterranean region and has been known for its medicinal properties since ancient times. Olive leaves and their extracts are used for a number of different purposes, such as to provide nutrients, control weight loss and help fighting against a variety of illnesses. It was found to produce greater weight loss in breast cancer survivors compared to a more traditional low-fat diet (Flynn and Reinert, 2010).

Olive oil extracted from *Olea europaea* has been widely used in traditional medicine for several thousands of years in countries of the Mediterranean basin. Coronary heart disease and colonic and prostate cancers were found to be very low in the Mediterranean countries (Keys, 1995; Hu, 2003). Based on the importance of olive oil in nutrition, many researchers have searched for olive

sub-products that could have beneficial effects on human health. In this context, compounds obtained from OLE have been subject to numerous investigations. OLE has been commercialized as a food supplement which can be consumed in the form of tea, syrup and capsules.

OLE is well-known for its antioxidant properties, hypotensive, hypoglycaemic and cardiovascular, radio- and hepato-protective effects (Benavente-Garcia *et al.*, 2002; Khayyal *et al.*, 2002; Komaki *et al.*, 2003; Somova *et al.*, 2004; Bouaziz and Sayadi, 2005; Poudyal *et al.*, 2010). It was also known for its antimicrobial activity and anti-inflammatory properties (Bisignano *et al.*, 1999; Lee-Huang *et al.*, 2003; Micol *et al.*, 2005; Bitler *et al.*, 2005). Olive leaves contain secoiridoids such as oleuropein, ligostrósíde, dimethyl oleuropein and oleoside, flavonoids, including apigenin, kaempferol and luteolin as well as phenolic compounds such as caffeic acid, tyrosol and hydroxytyrosol (Chiou *et al.*, 2007; El and Karakaya, 2009).

Although, these properties have been attributed to the different constituents of the plant leaves, there is minimal data available which concerns the correct dosage required to achieve the previously described health benefits and if there are any side effects from the long term intake of the OLE. This study was undertaken to evaluate the possible haematological, biochemical as well as histological alterations which may result from intake of OLE in Wistar rats which is used as natural therapy.

MATERIALS AND METHODS

Olive leaves and extracts: Olive leaves were collected from Tabuk region of Saudi Arabia. The leaves were shade dried and were crushed to moderately coarse powder. A weight of 100 g of shade-dried leaves was ground in an electrical grinder and dissolved in 500 mL distilled water. The suspension was thoroughly mixed and was left stirring using magnetic stirrer for 24 h. The next day the mixture was strained out using fine sieve and the crude extract was air evaporated for three days. The concentrated OLE for the plant was then orally administered to rats by gastrogavage.

Experimental design: Thirty, male Wistar white rats weighing 300-340 g were obtained from animal facility at the College of Pharmacy, King Saud University and kept at the animal house at the Department of Zoology, College of Science, King Saud University for acclimatization. Rats were fed on standard laboratory chow and had free access to water under well ventilated conditions of 12 h day light cycle. The animals were acclimatized to laboratory conditions for 7 days prior to the commencement of the experiment. The rats were assigned at random to 5 groups each composed of 6 rats. Group 1 rats served as controls and were fed on the control diet. OLE extract was mixed with diet and administered by gastrogavage at concentrations of 0.2% (group 2), 0.4% (group 3), 0.7% (group 4) and 0.9% (group 5) everyday for 6 weeks. The general health condition of the animals was monitored daily throughout the experimental period.

Haematology, biochemistry and histopathology laboratory work: At the end of experiment, blood samples collected from the retro-orbital plexus using a glass capillary tube into two sets of blood tubes one with anticoagulant (EDTA) for haematological tests and the other one without anticoagulant added for biochemical investigations. The Haematology analyser CCP Beckman counter (Beckman Coulter, California, USA) was used to determine the complete haemogram of the experimental animals. Parameters determined included White Blood Cell Counts (WBCs), Red Blood Corpuscles Counts (RBCs), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV),

Haemoglobin (Hb) concentrations and Mean Corpuscular Haemoglobin Concentrations (MCHC). Sera were analyzed for the activities of Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) and for the concentrations of, total bilirubin, cholesterol, triglycerides and glucose using the biochemistry analyser BSA 3000 (Diagnostic Instruments Manufacturer, SFRI France). Animals were killed using a high dose of isoflurane, representative samples of the livers, kidneys and spleens of the experimental animals were fixed in 10% neutral buffered formalin and processed for histopathology and stained with haematoxylin and eosin.

Statistical analysis: Statistical analysis was assessed using student t-test using the computer program Sigmastat (Sigmastat Statistical Software, version 2.03, SPSS Inc). A value of $p \leq 0.05$ was considered significant for statistical tests.

RESULTS

No clinical abnormalities were observed from the experimental animals throughout the course of the experiment. Animals did not show any signs of sickness and there was no obvious clinical manifestation like weakness, diarrhoea or yellow discoloration of the mucous membranes.

The haematological findings were shown in Table 1. There was significant decrease in Hb, RBCs, PCV, MCHC in group 5 compared to the control group ($p \leq 0.05$). The other groups did not show significant differences in the erythrocytic series. However, group 2 showed significant decrease in both the HB and the PCV compared to the control group ($p \leq 0.05$). The MCV was significantly higher in groups 3, 4 and 5. The WBCs showed marked reduction in the test groups compared to the control group.

Biochemical values of experimental animals are tabulated in Table 2. It was noticed that there was significant increases in the levels of both total bilirubin and ALP of the experimental animals

Table 1: Haematological values of Wistar rats fed olive leaves extract for 6 weeks

Parameters	G1 (control)	G2 (0.2%)	G3 (0.4%)	G4 (0.7%)	G5 (0.9%)
RBC ($\times 10^{12} L^{-1}$)	14.7 \pm 2.6	15.3 \pm 2.7	14.2 \pm 2.6	13.5 \pm 0.6	13.1 \pm 0.3*
PCV (%)	52.3 \pm 2.6	49.2 \pm 3.4*	53.6 \pm 4.1	53.1 \pm 2.0	49.2 \pm 1.1*
Hb (g dL ⁻¹)	11.4 \pm 2.9	9.7 \pm 0.1*	11.3 \pm 1.7	11.0 \pm 0.0	9.5 \pm 2.6*
MCH (pg)	7.9 \pm 2.6	6.3 \pm 1.31	8.7 \pm 2.2	8.4 \pm 0.1	8.5 \pm 0.7
MCHC (g dL ⁻¹)	20.9 \pm 1.4	19.6 \pm 0.6	21.0 \pm 2.2	20.1 \pm 0.2	19.2 \pm 2.5*
MCV (fl)	35.8 \pm 8.1	34.9 \pm 3.4	40.8 \pm 11.6*	39.5 \pm 0.1*	37 \pm 1.1*
WBC ($\times 10^9 L^{-1}$)	19.5 \pm 2.7	16.2 \pm 5.4*	17.7 \pm 4.2*	15.7 \pm 3.9*	17.2 \pm 3.1*

RBC: Red blood cells, PCV: Packed cell volume, Hb: Haemoglobin concentration, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, MCV: Mean corpuscular volume, WBC: While blood cells, *Significant at $p \leq 0.05$

Table 2: Biochemical values of Wistar rats fed olive leaves extract for 6 weeks

Parameter	G1	G2	G3	G4	G5
Total bilirubin (mg dL ⁻¹)	0.6 \pm 0.1	0.7 \pm 0.2	5.7 \pm 7.2**	5.9 \pm 0.9**	7.2 \pm 8.9**
Alkaline phosphatase (IU L ⁻¹)	102.3 \pm 2.5	318 \pm 2.1**	406.5 \pm 5.1**	423.6 \pm 4.9**	650.1 \pm 3.2 **
Lactate dehydrogenase (IU L ⁻¹)	314.1 \pm 101.1	321.4 \pm 262.5	349.7 \pm 102	265.1 \pm 64.5	482.4 \pm 158.9**
Cholesterol (mmol L ⁻¹)	2.6 \pm 0.4	2.4 \pm 0.6	2.1 \pm 0.6	1.9 \pm 0.7*	1.6 \pm 0.6*
Triglycerides (mmol L ⁻¹)	1.0 \pm 0.1	1.2 \pm 0.1	0.9 \pm 0.1	0.8 \pm 0.05	0.8 \pm 0.2
Glucose (mmol L ⁻¹)	5.3 \pm 1.5	5.1 \pm 1.4	5.7 \pm 1.1	4.6 \pm 2.0*	4.1 \pm 0.6*

***Significant at $p \leq 0.05$ and $p \leq 0.001$, respectively

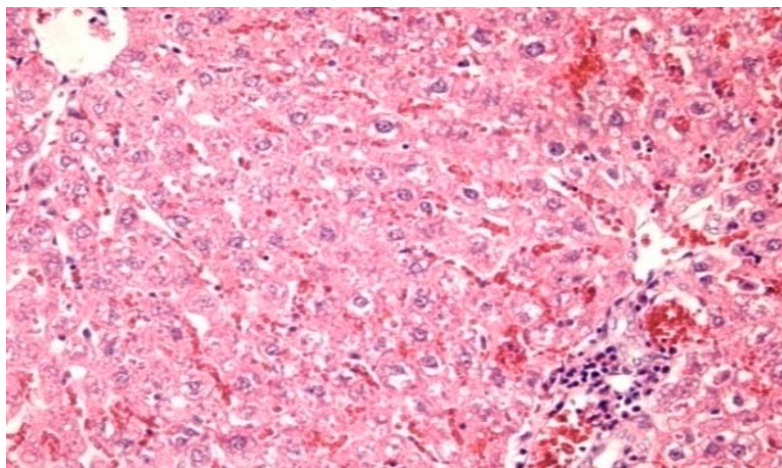


Fig. 1: Histological section of a liver from rats which were fed basal diet (control) for 6 weeks (section stained with H and E X200)

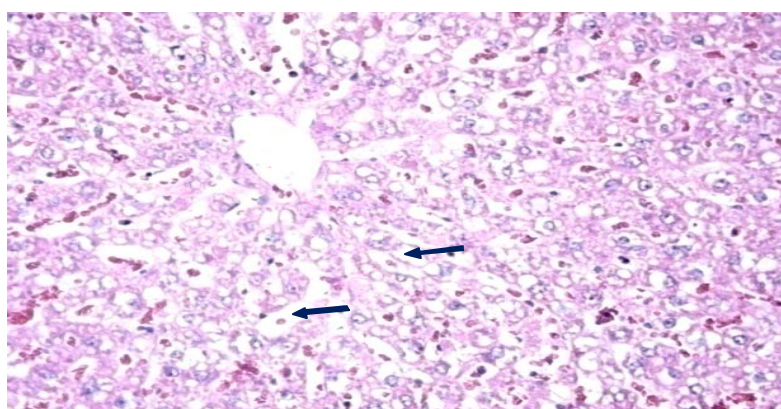


Fig. 2: Histological section of a liver from rats which were fed diet containing 0.9% OLE for 6 weeks showing fatty change and hepatocellular necrosis. Arrows show empty areas as an indication of fatty change and some of the nuclei showed degeneration (section stained with H and E X200)

compared to the control group ($p \leq 0.001$), with the exception of the level of total bilirubin in group 2 which was not found to be significant. The LDH was increasingly significant in group 5 ($p \leq 0.001$) while animals in other experimental groups showed no significant increase in the serum levels of LDH. Cholesterol and glucose were significantly lower in both group 4 and 5 ($p \leq 0.05$) compared to the control group.

No histological changes showed in liver (Fig. 1) of control group. However, liver of individuals in group 4 and 5 showed fatty change as well as hepatocellular necrosis which was mild in livers of individuals from groups 2 and 3 (Fig. 2). The kidney tissues of the control group remained without histopathological changes (Fig. 3), whereas sections of kidneys from the test groups showed streaky haemorrhages and congestion in the cortical region

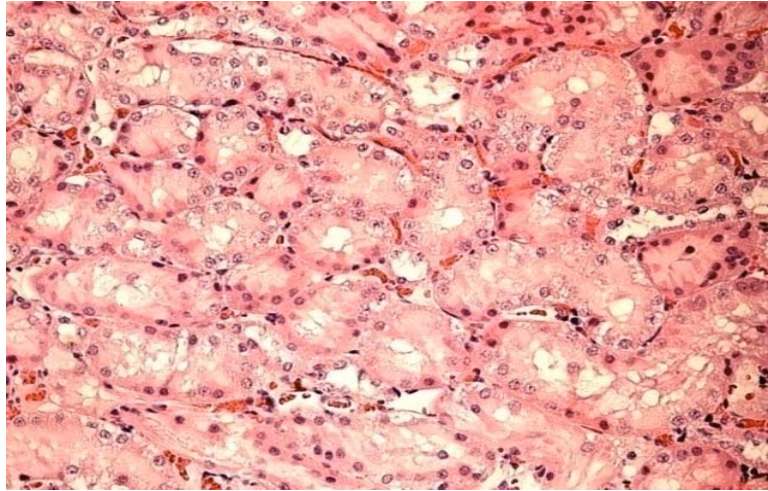


Fig. 3: Histological section of a kidney from rats which were fed basal diet (control) for 6 weeks (section stained with H and E X200)

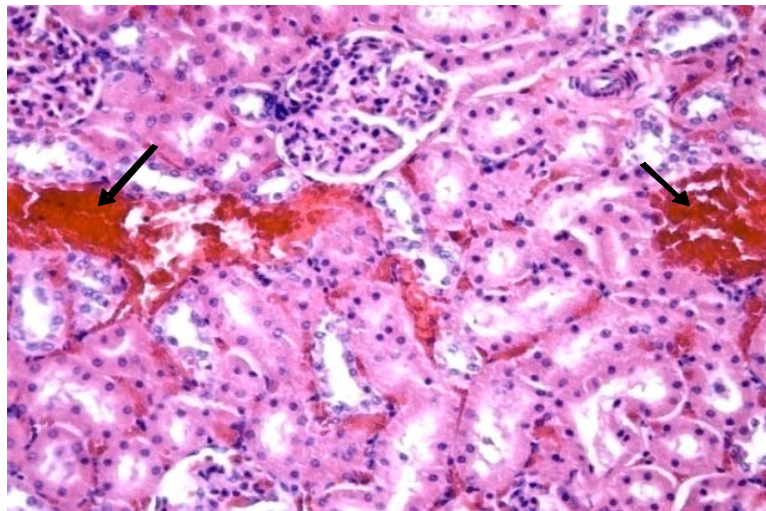


Fig. 4: Histological section of a kidney from rats which were fed diet containing 0.9% OLE for 6 weeks showing cortical haemorrhages. Arrows point at areas with massive haemorrhage and congestion (section stained with H and E X200)

and haemorrhage was more severe in animals in groups 4 and 5 (Fig. 4). Spleen did not show pathological changes in both the control group and the test groups.

DISCUSSION

Olive leaves extracts is generally available commercially and it was used without prescription and people use it for the treatment of various ailments. The effect of long term usage of OLE has

not been seriously investigated. A part from the report by Arantes-Rodrigues *et al.* (2011) showed that no report has indicated the negative effects of OLE over longer periods of time (Arantes-Rodrigues *et al.*, 2011). In the present study we have shown that feeding doses of 0.2-0.9% of OLE to Wistar rats for a period of 6 weeks may adversely affect the animals fed OLE. This was indicated by the haematological, biochemical as well as histopathological changes in the livers and kidneys of the experimental animals.

OLE at the dose level of 0.9% for 6 weeks induced significant changes in the haematological values of the experimental animals. The anaemia in the form of decreased RBCs, Hb and PCV in animals fed 0.9% OLE may have resulted from the loss of blood as supported by histopathological changes indicated in the kidneys. It is difficult to explain the significant decrease in the Hb and PCV in animals fed 0.2% OLE and not in those animals fed 0.4 and 0.7% OLE. However, the decrease in the RBCs, HB and PCV in group 5 which were fed 0.9% OLE appeared to be dose related.

Increased levels of ALP and LDH and of serum total bilirubin indicated hepatocellular damage which increase proportional to the number of hepatocytes affected (Amacher, 1998; Kew, 2000). Similarly in earlier two studies, levels of ALT, LDH and total bilirubin were reported to have increased in rats as a result of being fed high doses OLE and phenolic compound of olive for 14 weeks and 7 weeks respectively (Farag *et al.*, 2003; Arantes-Rodrigues *et al.*, 2011). In agreement with our investigation the histological changes reported in the liver were found to be dose dependant (Arantes-Rodrigues *et al.*, 2011). Contrary to ours and Arantes-Rodrigues *et al.* (2011) and Farag *et al.* (2003) reported no histological changes in the livers of the experimental animals. Increased levels of total bilirubin in serum encountered in the present study as a result of feeding OLE for 6 weeks may be indicative of liver impairment a fact that has been documented previously by Engelking (1988) and Anderson and Washabau (1992). The reduction in serum glucose in the experimental animals suggested hyperglycaemic effect of OLE which has been demonstrated earlier by Gonzalez *et al.* (1992) and Khayyal *et al.* (2002). The hypocholesterolemic effect of OLE in experimental animals coincided with what has been published earlier (Perrinjaquet-Moccetti *et al.*, 2008; Poudyal *et al.*, 2010). The reduction in the blood lipid concentrations could be due to agonist actions on bile acid activated TGR5, a metabotropic G-protein-coupled receptor (Sato *et al.*, 2007).

CONCLUSION

Feeding olive leaves extracts to Wistar albino rats for 6 weeks at a dose up to 0.9% resulted in hepatocellular and renal abnormalities. Olive leaves extracts in the present study, however, has resulted in lowering cholesterol and blood glucose. Therefore, further work is needed to determine the correct dose, to be used over long time which results in minimal or no pathological changes and at the same time continue to reducing blood sugar and cholesterol.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-033.

REFERENCES

Amacher, D.E., 1998. Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. *Regul. Toxicol. Pharmacol.*, 27: 119-130.

- Anderson, J.G. and R. Washabau, 1992. Icterus. *Comp. Cont. Educ. Pract. Vet.*, 14: 1045-1061.
- Arantes-Rodrigues, R., A. Henriques, M.J. Pires, B. Colaco and A.M. Calado *et al.*, 2011. High doses of olive leaf extract induce liver changes in mice. *Food Chem. Toxicol.*, 49: 1898-1997.
- Benavente-Garcia, O., J. Castillo, J. Lorente and M. Alcaraz, 2002. Radioprotective effects *in vivo* of phenolics extracted from *Olea europaea* L. leaves against X-ray-induced chromosomal damage: Comparative study versus several flavonoids and sulfur-containing compounds. *J. Med. Food*, 5: 125-135.
- Bisignano, G., A. Tomaino, R.L. Cascio, G. Crisafi, N. Uccella and A. Saija, 1999. On the *in vitro* antimicrobial activity of oleuropein and hydroxytyrosol. *J. Pharm. Pharmacol.*, 51: 971-974.
- Bitler, C.M., T.M. Viale, B. Damaj and R. Crea, 2005. Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. *Nutr. Immunol.*, 135: 1475-1479.
- Bouaziz, M. and S. Sayadi, 2005. Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. *Eur. J. Lipid Sci. Technol.*, 107: 497-504.
- Chiou, A., F.N. Salta, N. Kalogeropoulos, A. Mylona, I. Ntalla and N.K. Andrikopoulos, 2007. Retention and distribution of polyphenols after pan-frying of French fries in oils enriched with olive leaf extract. *J. Food Sci.*, 72: 574-584.
- El, S.N. and S. Karakaya, 2009. Olive tree (*Olea europaea*) leaves: Potential beneficial effects on human health. *Nutr. Rev.*, 67: 632-638.
- Engelking, L.R., 1988. Disorders of bilirubin metabolism in small animal species. *Comp. Cont. Educ. Pract. Vet.*, 10: 712-716.
- Farag, R.S., G.S. El-Baroty and M. Basuny, 2003. Safety evaluation of olive phenolic compound as natural antioxidants. *Int. J. Food Sci. Nutr.*, 54: 159-174.
- Flynn, M.M. and S.E. Reinert, 2010. Comparing an olive oil-enriched diet to a standard lower-fat diet for weight loss in breast cancer survivors: A pilot study. *J. Women's Health*, 19: 1155-1161.
- Gonzalez, M., A. Zarzuelo, M.J. Gamez, M.P. Utrilla, J. Jimenez and I. Osuna, 1992. Hypoglycemic activity of olive leaf. *Planta Med.*, 58: 513-515.
- Hu, F.B., 2003. The Mediterranean diet and mortality-olive oil and beyond. *New Engl. J. Med.*, 348: 2595-2596.
- Kew, M.C., 2000. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet*, 355: 591-592.
- Keys, A., 1995. Mediterranean diet and public health: Personal reflections. *Am. J. Clin. Nutr.*, 61: 1321S-1323S.
- Khayyal, M.T., M.A. El-Ghazaly, D.M. Abdallah, N.N. Nassar, S.N. Okpanyi and M.H. Kreuter, 2002. Blood pressure lowering effect of an olive leaf extract (*Oleo europaea*) in L-NAME induced hypertension in rats. *Arzneimittel-Forschung/Drug Res.*, 52: 797-802.
- Komaki, F., E. Yamaguchi, S. Maru, M. Kinoshita, K. Kakehi, Y. Ohta and Y. Tsukada, 2003. Identification of anti-alpha-amylase components from olive leaf extracts. *Food Sci. Technol. Res.*, 9: 35-39.
- Lee-Huang, S., L. Zhang, P.L. Huang, Y.T. Chang and P.L. Huang, 2003. Anti-HIV activity of Olive Leaf Extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochem. Biophys. Res. Commun.*, 307: 1029-1037.
- Micol, V., N. Caturla, L. Perez-Fons, V. Mas, L. Perez and A. Estepa, 2005. The olive leaf extract exhibits antiviral activity against Viral Haemorrhagic Septicaemia Rhabdovirus (VHSV). *Antiviral Res.*, 66: 129-136.

- Perrinjaquet-Moccetti, T., A. Busjahn, C. Schmidlin, A. Schmidt, B. Bradl and C. Aydogan, 2008. Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces blood pressure in borderline hypertensive monozygotic twins. *Phytother. Res.*, 22: 1239-1242.
- Poudyal, H., F. Campbell and L. Brown, 2010. Olive leaf extract attenuates cardiac, hepatic and metabolic changes in high carbohydrate-, high fat-fed rats. *J. Nutr.*, 140: 946-953.
- Sato, H., C. Genet, A. Strehle, C. Thomas and A. Lobstein *et al.*, 2007. Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea*. *Biochem. Biophys. Res. Commun.*, 362: 793-798.
- Somova, L.I., F.O. Shode and M. Mipando, 2004. Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. *Phytomedicine*, 11: 121-129.