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DETERMINATION AND CONCENTRATION OF *OLEA EUROPAEA* LEAVES INGREDIENTS AND ITS EFFECT ON GLUCOSE INDUCED DIABETES AND SOME BLOOD BIOCHEMICAL STANDARDS IN MALE RATS

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ABSTRACT : This study was conducted in the Animal House, Laboratories of Biology Department, College of Education for Women, Tikrit University and Ministry of Science and Technology and Laboratory. The study aimed toDetermain kind and Concentration of *Olea europaea*ingredients by HPLC teqnique and effectits extract in glucose and some blood serum biochemical properties in male rats, the treatment were control, treatment with alloxane, treatment with alloxane + 1ml extract of the leaves of the olive treatment alloxane +2ml of the olive leaf extract. The results showed there were four ingredients on the plant leaves: Ferulic acid, Oleuropen, Tyrosol and Hydroxy-Tyrosel by 7.43%, 67.14, 15.72% and 9.70% respectively. Also significant increasing on the concentrations of blood glucose and cholestrol on the treatment with alloxane, while the concentrations of blood sugar and cholestrol were significantly reduced by the treatment with olive leaves extract on compare with the alloxane treat. The concentrations of high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein- cholesterol (VLDL-C) in the male rats group injected with alloxan were significantly increased ($p \leq 0.05$), while the (HDL-Cand(VLDL-C) increased significantly in the the treatment with plant extract and the rats with high of olive leaf extract, while LDL-C reducedwith (alloxan +plant extract).

Key words : Medicinal plants, olive extract, diabetes cholestrol lipoproteins.

INTRODUCION

Plant kingdom concedered wonderful kingdom, human were used more of them as food and some other as medicine and so the human searched and get acquainted on more of plants characteristics and so he recognizes on what is useful and harmful from wild plant (Bennett and Plum, 2004) and most of this plant used for treatment Cases of diarrhea, gas repellent, treatment of diabetes, cough and other diseases. And as a result of the resulting damage of using industrial medical drugs and its side effect this push human to attention on natural plants as natural drug. In terms of their use as a naturally hand -made drug and thus reducing the damage on human life and be in more effective than laboratory prepared materials and its available chep than industrial drugs where many patients faces frequent costs in most poor countries (Abo-Zaid, 1986), many studies appeared positive effect of mant extracts of medicinal plants and herbson on reducing blod sugar levels on tested animals (Chakrabarti *et al*, 2003).

Studies have shown that olive tree has great economic benefits and to her leaves several mecinal effects (Panizzi, 2010) and at the beginning of the twentieth century extract the most important compound from olive leaves is oleuropen and then through studies appeared this compound lead to reduce blood pressure (Zarzuela, 1999). Leaves extract is one of the best treatments which used for reducing sugar level on blood and maintain its level within the normal range after eating and this extract is not allowed using with diabetes drugs where olive leaves contain oleuropen compound, which turns to calcium nulateand responsible for reducing the spread of germs and viruses, also it prevent chollestrol oxidation process type (LDL) (Rose and Lynn, 2001) and the scientists refer to rolr of olive compounds on treat blood pressure cases, cholesterol diabetes and as antioxidant (Samuelsson, 2011).

Diabetes is disorder process of hormones and lack of balance on sugars, lipids and proteins metabolism and rise sugar level of blood as aresultInsulin deficiency or a bug in the insulin process or on both (Jayasri *et al*, 2008; Tenpe and Yeole, 2009). The infection by diabetes due to many causes as partialy difficit on insulin hormone

production (Webster, 2004; Barnbek *et al*, 2011), blocking the function of beta cells because of oxidation stress (Petit and Adamec, 2011).

The substance aloxane is one of toxic substances for beta cells in pancreas drived from uric acid which uses on urgging diabetes on laboratory animals (Abu Abeeleh *et al*, 2009). Ansulin hormone is responsible of control and regulation processes of sugar level on blood, there are several methods of treatment of Diabetes Mellitus such as medication given by glaucoma, as medication given by the mouth and Herbal Therapy (Haslett *et al*, 2002).

Cholestrol is fatty and waxing substance at the same time and it will be on two types, High–density lipoprotein (HDL) and Low–density lipoprotein (LDL) which known as the beneficial protein or good for health as its percentage should be (40mg/dl), while the second type is Low–density lipoprotein (LDL) which known as the bad protein or harmful for health as its percentage should be (100mg/dl), besides of very Low –density lipoprotein (LDL). The overall level of total cholestrol it must not exceed (200mg/dl) (Stryer, 2000).

MATERIALS AND METHODS

This study was conducted in the Animal House and in the Laboratories of Biology Department, College of Education for Women. The study aimed to determain kind and concentration of olive leaves ingredients by HPLC (high performance liquid chromatography) and effect its extract in glucose and some blood serum biochemical propertiesin male rats at 3-4 mounth age with 250-300gm weghit, the treatment were control, treatment with alloxane, treatment with alloxane +1ml extract of the leaves of the olivetreatment alloxane +2ml of the olive leaf extract.

Active plant ingredients

Diagnosis of plant active ingrediants done by High Performance Liquid Chromotography (HPLC) apparatus. The plant dried at the shade and milled then 10gm from the sample put in 50 ml boiled water (90-100^{0}C) for 3 hours then extracted Whattman papers no. 1 the extraction collected and put in closed glass tube in order measuring the concentration of active ingriadents by HPLC apparatus which supplied by Shimadzu company (Japan) type, LC-10A2000 supplied with spectrum scale (Spectro photo meter – spd – 10A – UV), A sample size 20µl injected on Fast liquid chramotographic column (LC) with diamention (50×4.6mm I.D) by the injector type (Rheodyn-712) and the data recorded by calculator, which drawed the pick area and retention time. A standard solution of *Olea europaea* plant used and sperated by HPLC apparatus and identification the pick area and retention time of standard solution and comparing it with the pick area and retention time of studed plant sample at the same condition (Nishizuw *et al*, 1991) (Table 1).

Table 1 : Chromatographics separate condition.

Col Column Revers phase column (50×2.0 mm I.D)
MoMobile phase 1% acetic acid : acetonitrol : methano l6:3:1v/v
Fol Following rate 1.2ml/min
Type of detector Ultra violate ray 282 nm
Tmperature 20^{0}C
Fast of recorder paper on the computer 10 ml/min
Size of injected sample 3µg/ml

Concentration of compounds in the sample calculated by the aquation

Conc. Compound in the sample =

$$\frac{\text{Pick area of compound}}{\text{Pick area of standard sample}} \times \text{standard pattern conc.} \times \text{dilution factor}$$

Preparing plants extracts : Leaves of *Olea europaea* leaves were washed by water and soaked in 2% of sodium hypochloride solution for 15 minut and washed with sterilized water and air dried at room temperature, 500gm of leaves milled and used for extraction in 100ml of hot water and the extraction dried by using water path at 60^{0}C inorder obtain 50gm dried extraction then 1and 2ml prepared from the dried extraction (Al-Janabi *et al*, 2015)

Biochemical blood parameters

Weight male rateIn this study used at age ranged between 4-3 mounth with 250-300 gm weight. For the inducing of experimental diabetes by injection them under the skin (Subcutaneous) with alloxan substance from British BDH company, where it was prepared at injection time by dose 150mg/kg from body weight by desolving 1gm from alloxan substance on 10cm^3 from physiology saline solution.

Afterend specified period 60 days and after inducing sugar diabetes in the blood on male rate and after giving plant extract for 60 days, animal hungered and Blood was pulled once every two weeks and the number of wanted pulls were four in the experiment, the blood pull from Vein located in eye angle by using red hairy tubes empty from anticoagulant, then serum sperated by centerifuge appraratus with speed 3000 r/m for 5 minutes and the serum kept in the fridge at 20^{0}C until conduct the laboratory tests.

Determination of Serum Glucose level glucose measured by using equipment test (kit) produced by Bio

system USA company according the method, which depends to enzymatic analysis of glucose by glucose oxidase where it consists hydrogen peroxide, which is detected by 4-Amino-antypyrine solution with existing peroxidase and as a result of this will appear pinky coulor it results from being Quinonimine substance, by taken three test tube and putting on each one $1cm^3$ from working solution composed of Enzyme Reagent (R_2), which consist of Glucose oxidase 10000 u/L, peroxidase 1000u /L and 4-amino- antipyrine 2.6 mmol/L with same size from reagent buffer (R_1) consist of Tris buffer (PH 7.0), 100 mmol / L and Phenol 0.3 mmol/L and the two solutions shaked it lightly and leave 10 minute at laboratory temperature and absorption was on wave length 505nm using aquation below:

$$\text{Glucose conc. (mg/dl)} = \frac{\text{A. sample}}{\text{A. standard}} \times \text{Standard Conc. STD}$$

A = Absorptin

STD = 100mg/dl

Cholestrol estimation

Cholesrol measured by using equipment test (kit) produced by Bio system USA company according the method which depends to enzymatic analysis of cholesterol by cholesterol oxidase intopinky pigment Quinonimine, by taken three test tube and putting on each one $1cm^3$ from working solution composed of enzymatic reagent ((R2) which consist of (Peroxidase 1250µ/L, Cholestroloxidase 300, Cholestrol esterase 300 and Aminophenozone 0.4mmol/L) with same size from Regent buffer (R1) composed of (Pipes(Ph 6.9), 90mmol/ L Phenol 20 mmol/L)) and the two solutions shaked and produced solution be fixed to for 40 days at temperature 15-25°C or three mounth at 2-8°C then added 10 µ/L from blood serum to one test tube and 10 from standard solution (R3) consist of (cholesterol 200mg/dl) to second tube prepar working solution, then taked three test tube and put in each one 1.0 ml from working solution then addition 10µ from blood serum on one of tubes then shaked it lightly and leave 5 minute at temperature 37°C. The reading was on wave length 500nm using aquation below:

$$\text{Cholestrol conc.(mg/dl)} = \frac{\text{A. sample}}{\text{A. standard}} \times \text{Standard Conc. (n)}$$

A = Absorption,

n = (200mg/dl or 5.17 mmol/L)

Determination of Serum Triglyceride (TG) level

Triglyceride level measured by using equipment test (kit) produced by (Giesse Diagnesticsns) Italy company, this method depend on base enzymatic analysis of triglyceride into glycerolby taking three test tube and add 10 from blood serum, standard solution (Glycerol 200mg/ dl) and distile water on the tube respectively, then it was added 1 ml from detergent solution (TG) on each tube, then leaved on laboratory temperature after shaking lightly of:

$$\text{Triglycerides Concentration (mg/dl)} = \frac{\text{A. Sample}}{\text{A. Standard}} \times \text{Standard Conc.}$$

Standard Concentration = 200mg/dl or × 0.0113 mmol/ L

A = Absorbance

Determination of Serum HDL-C level

HDL-C measured by using equipment test (kit) produced by Bio system USA company according enzymatic method depeding on quantity sedimentation of low density lipoprotein LDL, VLDL. The leachate which obtained after separation process by using centerfuge contain only HDL and it measured by using cholesterol enzymative solution, the HDL measuring as below:

$$\text{HDL conc.(mmol/1)} = \frac{\text{A. sample}}{\text{A. sample}} \times \text{Standard Conc}$$

Standard Conc = 1.29 mmol/l

Determination of Serum LDL-C level

It measured on the blood serum by the aquation : LDL-C (mg/dl) = VLDL+HDL

Determination of Serum VLDL-C level

It measured depending on the following relationship: VLDL (mg/dl) = (Triglycerides/5).

The data analysed statisticaly by using complete Randomized Design (CRD) and least significant diffrenece compared between means by using Duncan multiple test (Duncan, 1955) at the leavel 5% by using statistical program (SAS, 2001).

RESULTS AND DISCUSSION

Analysis Standard solution of *Olea europaea* leaves by HPLC teqnique proved contain the solution severel compounds as Ferulic acid, pleuropen, Tyrosel and Hydroxy-Tyrosel (Fig. 1and Table 2), while the chemical analysis by the same teqnique of studied *Olea europaea* leaves appeared compounds as Ferulicacid, pleuropen, Tyrosel and Hydroxy-Tyrosel by percentage 7.43%, 67.14%, 15.72% and 9.70%, repectively (Fig. 2 and Table 3).

Chromotograhic is a method for separation and purification the different chemical materials (Wasf's and Maher, 1993) and the HPLC teqnique is fast method,

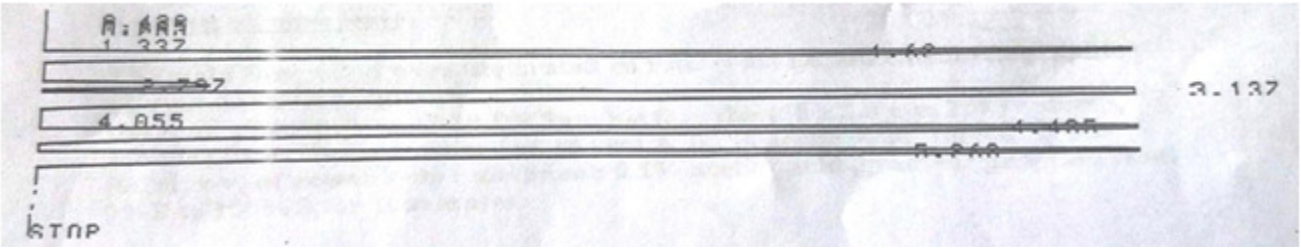

Fig. 1 : Pick area and retention time of standard.

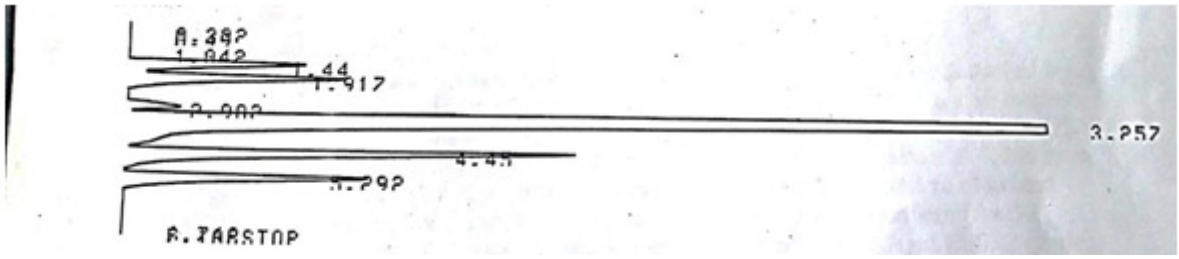

Fig. 2 : Pick area and retention time of *Olea europaea* leaves ingredients.

also its easy and keep on compounds quantity required to separate it and useful for separate any mixture whether was in solid, liquids gases state and another methods such as filtration, distilition and sedimentation led to lose a big part from water (Al-Aofe, 1991). HPLC teqnique proofed contain olive leaves to the benzoic as Galic acid, Caffeic acid, Vanilic acid and Synergic acid (Malo and Gaithaa, 2014), also HPLC and TLC teqnique showed contain oliv leaves many glycosides, phenols, resins, flavonoids and alkaloides compounds (Altaef, 2016). Our results agree with results of HIV (2003), whom refer to contain olive leaves on rich compounds with Oleuropen and Tyrosel also agree with study (Altýok *et al*, 2008), whom proeef through using Gos-chromotoghy and Mass-spctrometry teqnique contain olive leaves many compounds as Oleuropen.

From Table 4, verily alloxan substance has been updated increase on blood sugar concentration throughout the experiment and highest percentage at first and second week reached 535.0±141.6 mg/dl, while olive leaves extract proofed superior on reducing blood sugar percentage throughout the experiment and least percentage were at the treatment (alloxan+1 ml extract) at third and fourth week reached 107.71±13.3 mg/dl under test level ($p \leq 0.05$).

Incresing on alloxan concentration due to attacking aloxan for beta cells in pancreas and destraction it by accumulation free radicals, which concederd toxic topaccreasic beta cell swhat causes glucose accumulation and Blocking metabolism the glucose in this way man is affected with diabetes (Benrebai *et al*, 2007). The reducing on blood sugar by leaves extract and increasing on insulin secretion due to containing the extract many active compounds the most important one is Oleuropein (Ordonse *et al*, 2007) and the extract improve induction on increase insulin concentrations (Wainstein *et al*, 2012). The results agrees with Al-Chalabi *et al* (2009).

From Table 5, we see the treatment with alloxan led to icreasing on blood colestrol on all ages and the highest were (90.86±7.7) mg/dl at seventh and eighth week while the treatment (alloxan+ 1 ml) superior on reducing cholesterol percentage and gaved lower value reached (64.14±12.7) mg/dl at third and fourth week under test level (pH 0.05).

Introduction of diabetes by alloxan in male rate led to increasing cholesterol level on blood serum because of increasing on activity of Cholesterol –Acyl-transferes enzyme which responsible of cholesterol absorption on stomach, which stimulates on insulin hormone absence, also the infection with diabetes on male rates led to defector weak on kidney process and as result of this cholesterol concentration will increase (Chandramohan *et al*, 2009), verily using olive leaves extract led to reduce cholesterol concentration due to its containing several active compounds such as phenols and terpenes (Gordon, *et al*, 2001). Our study agree with Al-Mahamdy (2005) and Tenpeand Yeole (2009).

The results in Table 6 appeared verily highest TG concentration were at age seventh and eighth week on treatment with alloxan reached (133.00±14.1) mg/dl which did not differ from treatment with same material at ages third and fourth also fifth and sixth,while superior the treatment (alloxan+2 ml extract) on reducing TG percentage with value reached (100.00±9.0)mg/dl at the first and second week under test level ($p \leq 0.05$).

The reason of TG increasing due to loss of insulin, where it inhibits Lipoprotien lipase enzyme activity, which

Table 2 : The sequence of eluted material of the standard.

Compounds	Pick area	Retention time	Concentration (µgm/ml)
Ferulic acid	164704	1.62	25
Oleuropen	303765	3.13	25
Tyrosol	198801	4.43	25
Hydro–Tyrosol	192593	5.26	25

control where it reached the highest value (41.43±9.5) mg/dl on the treatment (alloxan+2 ml extract) at the fifth and sixth week under test level (pH 0.05). The decreasing on HDL-C level as a result of alloxan due to reducing on activity of Lipoprotein lipase enzyme also the increasing on Hepatic lipase enzyme activity where HDL-C became rich with TG and became important and essential materialon which the hepatic lipase enzyme works on it

Table 3: Compounds, pickarea, retention time and concentrationof studied plant.

Compounds	Pick area	Retention time (minute)	Concentration(µgm/l)	Concentration(%)	Delution factor
Ferulic acid	28133	1.614	213.514	7.431	50
Oleuropen	46880	3.257	1929.151	67.143	50
Tyrosol	71858	4.450	451.821	15.725	50
Hydro –Tyrosol	42941	5.292	278.703	9.700	50

Table 4 : Effect of Alloxan and *Olea europaea* leaves extract on blood sugar concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	96.57±22.9c	92.14±13.9c	97.00±14.3c	93.57±11.0c
Alloxan	535.0±141.6a	450.6±228.7a	500.1±135.9a	354.4±94.3a
Alloxan+1ml	139.71±21.1bc	107.71±13.3b	125.86±22.1bc	135.29±21.9c
Alloxan+2ml	115.86±19.2b	121.43±13.0b	161.71±21.0b	276.9±133.9b

*Different laters vertically means there are significant difference (p≤0.05).

Table 5 : Effect of Alloxan and *Olea europaea* leaves extract on blood cholesterol concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	53.43±3.41c	72.57±14.8b	82.43±10.4a	81.86±16.4b
Alloxan	84.00±10.0a	82.14±12.39a	77.29±7.30a	90.86±7.7a
Alloxan+1ml	74.43±13.6b	64.14±12.7c	79.57±14.4a	63.86±5.2c
Alloxan+2ml	70.86±12.7b	78.57±9.64ab	78.86±8.0a	83.29±7.2b

*Different laters vertically means there are significant difference(pd"0.05).

ply important and active role in the conversion process of TG to glycerol and fatty acids, which absorption by and through Intestinal cells (Nelson and Cox, 2005). Our study agree with Kim *et al* (2006) while disagree with Al-Afleh (2005) who see non significant increase on TG level. While Oral administration of infection rates on alloxan with plant extract led to increase on TG level because contain the extract important compounds as oleuropen which causes increasing activity of hepatic liver cells and insulin secretion (Jarald *et al*, 2008).

Treatment with alloxan were clear on increasing HDL-C concentration on all ages incompar with control, the highest value were reached (49.14±7.3) mg/dl when the rates treated with alloxan on seventh and eighth week and which did not differ significantly from other ages and the treatment with plant extracts led to increase HDL-C level significantly on compare with the treatment and as a result of this will led to speed up the process of HDL-L of the gyro device, which cause reduce its level on blood serum (Bilgin and [a] ahin, 2013). Either cause the rise by oleaeuropaea leaves due to ability of the extract to stimulating HDL-C molecular production by liver and intestinal cells (Dineshkuar *et al*, 2010). The study results agree with results what reached by BAYCýN *et al* (2007).

Table 8 shows the significant effect of treatment with alloxan on increasing LDL-C concentration on compare with control where the treatment with alloxan was given hghest level reached (75.74±7.5 mg/dl) at seventh and eighth week but didn't differ significantly with value of other ages, either treatment with plant extracts showed also significant effect on reducing LDL-C where the lowest value reached (56.86±9.9 mg/dl) on the treatment (alloxan+2 ml extract) at the first and second week under

Table 6 : Effect of Alloxan and *Olea europaea* leaves extract on Triglyceride concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	109.6±47.1bc	114.4±64.6a	127.43±21.9a	136.1±9.9a
Alloxan	121.14±12.1b	122.14±25.1a	129.29±13.8a	133.00±14.1a
Alloxan+1ml	148.43±19.6a	135.57±16.2a	138.86±17.6a	155.86±14.9a
Alloxan+2ml	100.00±9.0c	124.86±12.4a	121.86±11.3a	136.14±14.9a

*Different laters vertically means there are significant difference (p≤0.05).

Table 7 : Effect of Alloxan and *Olea europaea* leaves extract on HDL-C concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	31.29±5.4C	33.00±5.6A	34.57±8.9A	32.29±6.6c
Alloxan	44.00±8.6a	38.00±10.2a	42.57±6.4a	49.14±7.3a
Alloxan+1ml	35.14±10.3bc	37.29±6.0a	41.43±9.5a	36.14±10.1b
Alloxan+2ml	38.00±8.3ab	37.43±7.2a	35.71±5.2a	36.14±6.2b

*Different laters vertically means there are significant difference (p ≤ 0.05).

Table 8 : Effect of Alloxan and *Olea europaea* leaves extract on LDL-C concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	53.20±12.7b	55.89±16.2a	60.06±7.5b	59.51±24.2a
Alloxan	66.94±7.8a	62.43±8.8a	68.43±4.5a	75.74±7.5a
Alloxan+1ml	64.74±13.0a	64.40±5.5a	69.20±12.2a	67.31±9.3a
Alloxan+2ml	56.86±9.9b	62.40±7.6a	60.09±7.0b	63.37±8.5a

*Different laters vertically means there are significant difference (p ≤ 0.05).

Table 9: Effect of Alloxan and *Olea europaea* leaves extract on VLDL-C concentrations on male rates.

Treatments	Mean±Standard deviation			
	Week(1-2)	Week (3-4)	Week (5-6)	Week (7-8)
Control	21.91±9.4cc	22.89±12.9aa	25.49±4.3aa	27.23±1.7aa
Alloxan	24.22±2.4b	24.43±5.0a	25.86±2.7a	26.60±2.8a
Alloxan+1ml	29.60±4.0	27.11±3.2a	27.77±3.5a	31.17±3.0a
Alloxan+2ml	20.00±1.8c	24.97±2.4a	24.37±2.2a	27.23±2.9a

*Different laters vertically means there are significant difference (p ≤ 0.05).

test level (p≤0.05). The reason of LDL-C level due to decrese on lipo protein lipase enzyme activity, which led to non desolving of TG and occurrence transformation of most LDL-C on blood serum (Karen *et al*, 2002). Our study with agree with results of Daisy *et al* (2009). And the reducing on LDL-C on blood serum on compare with control return to act exist compounds on *Olea europaea* leaves extract such as oleuropin as antioxidant with ability on reducing cholesterol level and easing its metabolism and its naturly in this case happen reduce on LDL-C (Visioli *et al*, 2002).

The results in Table 9 shows significant effect of alloxan on VLDL-C concentration on compare with control treatment where highest value reached (26.60±2.8 mg/dl), which didn't differ significantly from values of third and fourth also fifth and sixth week and the treatment (alloxan+ 1 ml extract) gived highest value reached (31.17±3.0 mg/dl) at the seventh and eighth week which didn't compared significantly from ages third and fourth also fifith and sixth week under test level (p≤0.05). Verily the reduce on VLDL-C concentration due to reducing on Lipoprotein Lipiase enzyme activity which leads to occurrence increase on TG level and this cause at same the significant increase VLDL-C, while oral feeding with plant extract had significant effect on VLDL-C concentration and the highest value were (31.17±3.0 mg/dl) on the treatment (alloxan + 1 ml extract) at seventh and eighth week which did not differ

significantly from other ages and this because of effect of insullin on the liver and desolve of fats on lipid cells, there were correlation between fat dissoliving and increasing on free fatty production in liver (Ohno *et al*, 2000) and this introduction producing thirds lipids in liver and increasing lipids percentage on blood besides to production LDL and VLDL from liver (Coppack *et al*, 1994).

CONCLUSION

The results showed there were four ingredients on the plant leaves: Ferulic acid, Oleuropen, Tyrosol and Hydroxy-Tyrosel. Significant increasing on the concentrations of blood glucose and cholestrol on the treatment with alloxane, while the concentrations of blood sugar and cholestrol were significantly reduced by the treatment with olive leaves extract on compare with the alloxane treatment.

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