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RESEARCH ARTICLE

Hypolipidamic studies on Egyptian propolis and *Foeniculum Vulgare* on alloxan induced diabetic rats

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Abstract

This study was carried out to evaluate the effect of Egyptian propolis and *Foeniculum Vulgare* on hypolipidamic in diabetic rats.

Materials and methods: Diabetes was induced with Alloxan (148mg/Kg) intraperitoneal. Rats with blood glucose level less than 300 mg/dL were excluded. Animals were divided into 8 equal groups (n=80); except negative control rats, all groups inoculated with alloxan Group 1: negative control of normal rats. Group 2: positive control of alloxan injected rats. Group 3: rats were treated with 200 mg/ kg of propolis. Group 4: rats were treated with 400 mg/ kg of propolis. Group 5: rats were treated with 200 mg/ kg of *Foeniculum vulgare*. Group 6: rats were treated with 400 mg/ kg of *Foeniculum vulgare*. Group 7: rats were treated with (200 mg/ kg of *Foeniculum vulgare*+200 mg/ kg of propolis). Group 8: rats were treated with (400 mg/ kg of *Foeniculum vulgare*+400 mg/ kg of propolis). Rats were treated orally for 28 days.

Results: Data showed significant increase in serum levels of Cholesterol, LDL, VLDL and triglyceride and significant decreased levels of HDL were observed in the diabetic untreated animals.

Conclusion: Propolis and *Foeniculum Vulgare* possesses hypolipidamic activities.

Introduction:-

The Greek word "propolis" means to glue and it describes also the role of propolis to cement openings of the bee hive. Thus propolis is also named "bee glue" **(Bogdanov, 2012).** Propolis, a natural product is a resinous substance that honey bees (Apis mellifera) collect from tree buds, shrubs or other botanical sources. The main chemical classes present in propolis are flavonoids, phenolics and other various aromatic compounds and has been used extensively in folk medicine due to its several pharmacological properties **(Abdul-Hadi, 2014).**

Foeniculum vulgare was found to control the lipid parameters and glycated haemoglobin in diabetic rats **(Dongare, *et al*., 2012).** *Foeniculum vulgare* decrease the deposition of triglycerides in the fatty liver form and facilitates the flow of blood in the coronary arteries by preventing the deposition of lipids in the light of the coronary arteries by reducing serum and liver lipids. Fennel is an aromatic and medicinal plant nutrition which showed Hypolipidemic and Anti-Atherogenic and therefore can be used for the prevention of cardiovascular diseases **(Oulmouden *et al*., 2014)**.

It is an established fact that hyperlipidemia is a major risk factor to the development of atherosclerosis and its complications **(Kaur *et al*., 2002).** Besides fasting hyperglycemia, several organs develop complications in diabetes. Key organs in this respect include the kidney (nephropathy), blood vessels (atherosclerosis and microangiopathy) **(Guyton and Hall, 2002) (Salehi, *et al.,* 2010).** Several studies reported that propolis could decrease levels of blood glucose as well as modulate blood lipid and reduce atherosclerosis in patients with diabetes mellitus **(Matsui *et al.,* 2004; Fuliang *et al*., 2005 and El-Sayed *et al.,* 2009).**

Diabetes results into metabolic imbalances, such as; hyperglycemia and hyperlipidemia and the deviation from the normal physiology of many tissues **(Yue *et al*., 2003).** As the most important chemical weapon 'of bees against pathogenic microorganisms, propolis has been used as a remedy by humans since ancient times. Propolis is a sticky, resinous substance collected by honey bees from the sap, leaves, and buds of plants, and then mixed with secreted beeswax. Propolis has been used as a folk medicine in many countries from ancient times **(Ahuja, V. and Ahuja, A. 2011).**

Materials and methods:-

Experimental Animals:-

The 80 male albino rats (*Rattus rattus)* at average weight of (190±10) at the beginning of the experimental. Obtained from the Egyptian holding company for biological product and Vaccines were used as experimental animals.

Induction of diabetes-

The animals were fasted overnight. Diabetes was induced by single intraperitoneal (i.p) injection of alloxan monohydrate (148mg/kg) in sterile normal saline (0.9%). The diabetic state was determined 72 hours after alloxan administration through the tail, using the one touch ultra-glucometer (Glucodoctor). Weekly record of blood glucose level was taken afterwards.

Propolis:-

Was obtained from hives of royal bee company Cairo, Egypt. During spring and summer seasons of 2014.

Form of the agent:-

Bulk of glue like brownish material resulted from scrapping off the frames of bee hives.

Preparation:-

Propolis bulks were cut into small pieces and mixed with deionized water and shacked at 95 c° for 2 hours according to therapeutic dose. Then cooled to room temperature and centrifuged at 1500 r.p.m for 5 minutes to obtain the supernatant **(El-Akabawy *et al*., 2004).** This occurs in genetic engineering center Al-azhar University.

Foeniculum vulgare:

Foeniculum vulgare seeds were collected from the local market in Egypt and identified by its morphological and microscopically characters

Preparation:-

Foeniculum vulgare extracted by distilled water using soxhlet apparatus in physiology lab faculty of science al Azhar University.

Experimental design:-

The patch of animals was distributed into eight groups as the following:

Group 1 Control (C): negative control of normal rats, (n=10 Rats) rats of this group were neither treated nor injected by alloxan. Group 2 Diabetes Mellitus (DM): positive control of alloxan injected rats, (n=10 Rats) rats of this group were injected by alloxan 148 mg/kg intraperitoneal. Group 3 Diabetes Mellitus+ 200 propolis (DM+200Pro): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 200 mg/ kg of propolis. Group 4 Diabetes Mellitus+ 400 propolis (DM+400Pro): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 400 mg/ kg of propolis. Group 5 Diabetes Mellitus+ 200 *Foeniculum vulgare* (DM+200FV): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 200 mg/ kg of *Foeniculum vulgare*. Group 6 Diabetes Mellitus+ 400 *Foeniculum vulgare* (DM+400FV): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 400 mg/ kg of *Foeniculum vulgare*. Group 7 Diabetes Mellitus+200 propolis + 200 *Foeniculum vulgare* (DM+200Pro+200FV): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with (200 mg/ kg of *Foeniculum vulgare*+ 200 mg/ kg of propolis). Group 8 Diabetes Mellitus+400 propolis + 400 *Foeniculum vulgare* (DM+400Pro+400FV): Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with (400 mg/ kg of *Foeniculum vulgare*+ 400 mg/ kg of propolis).

Biochemical analysis:-

1- Estimation of serum triglycerides (TG) level (mg/dl):

Serum triglycerides were determined according to the method described by **Fossati and Prencipe (1982)** using kit from Elitech diagnostic Co. France.

Principle: The enzymatic determination of the triglycerides was according to the following reaction

$$\text{Glycerol} + \text{ATP} \xrightarrow{Glycerokinase, Mg+2} \text{Glycerol-3- phosphate} + \text{ADP}$$

$$2H_2O_2 + \text{4-Chlorophenol} + \text{4-Aminoantyprine} \xrightarrow{Peroxidase} \text{Quinoneimine} + 4H_2O + \text{HCL}$$

2- Measurement of serum total cholesterol (mg/dl) level :

Serum cholesterol level was determined according to the method described by **Allain *et al*. (1974)** using kit from Elitech diagnostic Co. France.

Principle: The total cholesterol is determined after enzymatic hydrolysis and oxidation. The quinonemine is formed from hydrogen peroxide and 4-Aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol} + O_2 \xrightarrow{Cholesterol\ oxidase} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{Peroxidase} \text{quinoneimine} + 4\ H_2O$$

3- Determination of low density lipoprotein-cholesterol (LDL-C) level (mg/dl):

The LDL-C calculations were conducted according to the formula of

Wieland and Seidel (1982).

LDL-C = Total cholesterol – (TG/5) – HDL-C

4- Estimation of serum high density lipoproteins-cholesterol (HDL-C) level (mg/dl):

Serum cholesterol level was determined according to the method described by **Burstein *et al*. (1970)** using kit from Elitech diagnostic Co. France.

Principle: Chylomicrons, very low density lipoproteins (VLDL) and Low density lipoproteins (LDL) of serum are precipitated by phosphotungstic acid and magnesium ions. After centrifugation, high density lipoproteins (HDL) are in the supernatant. Cholesterol included in this phase, is measured by the same enzymatic method used for total cholesterol**.**

Results:-

Total cholesterol shows a significant decrease ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with Propolis 400mg, Diabetics + treated with *F. vulgare* 200mg, Diabetics + treated with *F. vulgare* 400mg Diabetics + treated with (*F. vulgare* 200mg+Propolis 200mg) and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg), when compared with Positive control (Diabetics) as in table (1) & fig. (1) Where Mean and S.E was **(75.67±2.96)** in Positive control (Diabetics) and were **(44.2±3.42), (59.5±3.92) and (39±5)** in Control (Non diabetics), Diabetics + treated with *F. volgure* 400mg and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg) respectively.

Triglyceride shows a significant decrease ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with Propolis 400mg, Diabetics + treated with *F. vulgare* 200mg, Diabetics + treated with *F. vulgare* 400mg, Diabetics + treated with (*F. vulgare* 200mg+Propolis 200mg) and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg), when compared with Positive control (Diabetics) as in table (1) & fig. (2) Where Mean and

S.E was **(174±4.35)** in Positive control (Diabetics) and were **(72.2±5.07), (77.67±1.85) and (46.33±1.85)** in Control (Non diabetics), Diabetics + treated with Propolis 400mg and Diabetics + treated with *F. volgure* 200mg respectively.

Low density lipoprotein (LDL) shows a significant decrease ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with Propolis 400mg, Diabetics + treated with *F. vulgare* 200mg, Diabetics + treated with *F. vulgare* 400mg, Diabetics + treated with (*F. vulgare* 200mg+Propolis 200mg) and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg), when compared with Positive control (Diabetics) as in table (1) & fig. (3) Where Mean and S.E was **(20.33±2.02)** in Positive control (Diabetics) and were **(4.2±1.28) and (14.25±2.86)** in Control (Non diabetics) and Diabetics + treated with Propolis 200mg respectively.

High density lipoprotein (HDL) shows a significant increase ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with *F. vulgare* 200mg and Diabetics + treated with *F. vulgare* 400mg when compared with Positive control (Diabetics) as in table (1) & fig. (3) Where Mean and S.E was **(13±2.08)** in Positive control (Diabetics) and were **(25.8±2.41) and (35.75±5.28)** in Control (Non diabetics) and Diabetics + treated with *F. vulgare* 400mg respectively.

Very low density lipoprotein (VLDL) shows a significant decrease ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with Propolis 400mg, Diabetics + treated with *F. vulgare* 200mg, Diabetics + treated with *F. vulgare* 400mg, Diabetics + treated with (*F. vulgare* 200mg+Propolis 200mg) and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg), when compared with Positive control (Diabetics) as in table (1) & fig. (3) Where Mean and S.E was **(34.67±0.88)** in Positive control (Diabetics) and were **(14.2±0.97),**

(15.67±0.33) and (9.33±0.33) in Control (Non diabetics), Diabetics + treated with Propolis 400mg and Diabetics + treated with *F. vulgare* 200mg respectively.

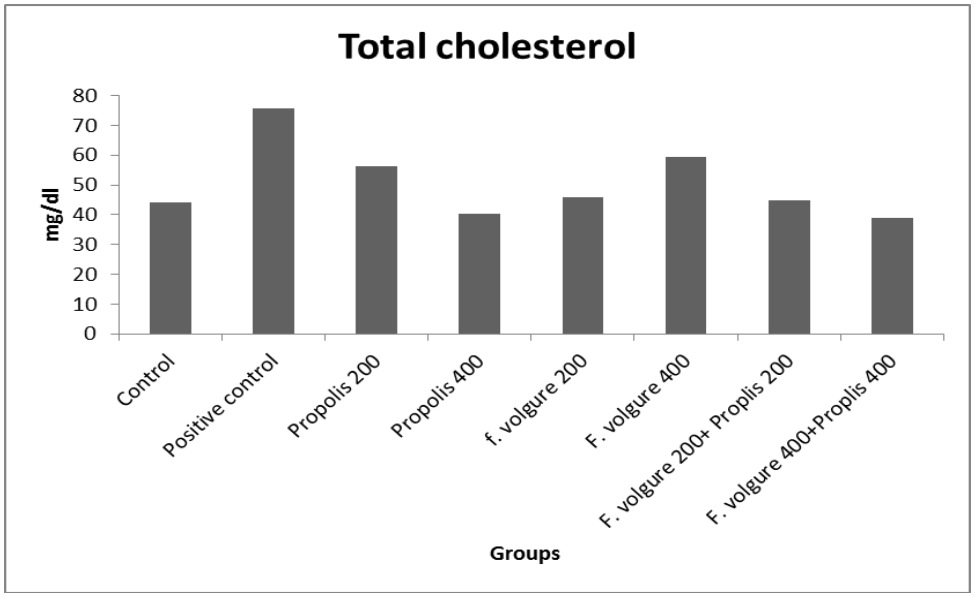

Fig (1): Shows the mean of total cholesterol concentration in rats subjected to alloxan and treated with Propolis & *F.volgure* doses for one month.

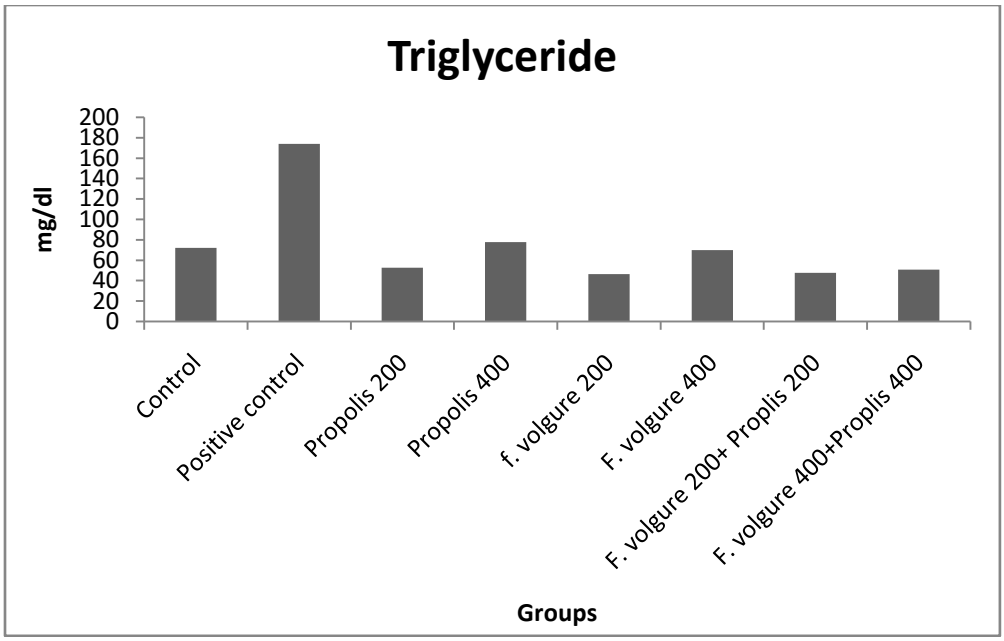

Fig (2): Shows the mean of triglyceride concentration in rats subjected to alloxan and treated with Propolis & *F.volgure* doses for one month.

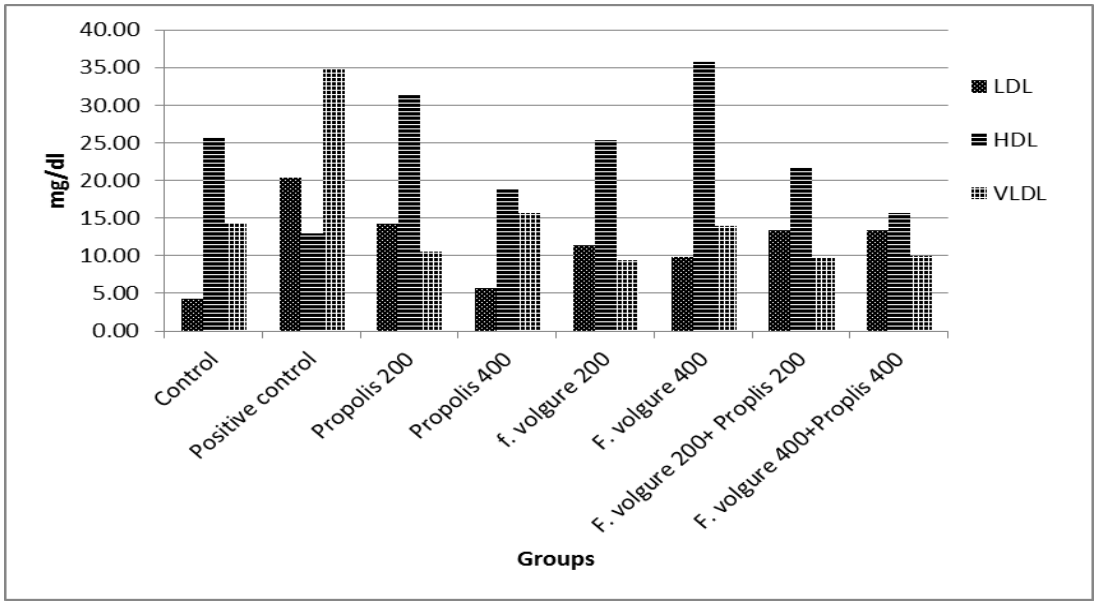

Fig (3): Shows the mean of LDL, HDL and VLDL concentration in rats subjected to alloxan and treated with Propolis & *F.volgure* doses for one month.

Table (1): Shows the mean ± SE of lipid profile concentration in rats subjected to alloxan and treated with Propolis & *F.volgure* doses for one month.

Parameters / Groups		Total cholesterol	LDL	HDL	VLDL	Triglyceride
C Non diabetic	**Means±SE**	**44.2±3.42a**	**4.2±1.28a**	**25.8±2.41a,c**	**14.2±0.97a**	**72.2±5.07a**
P.C Diabetic	**Means±SE**	**75.67±2.96b**	**20.33±2.02b**	**13±2.08b**	**34.67±0.88b**	**174±4.35b**
D+200P	**Means±SE**	**56.25±5.03c,d**	**14.25±2.86c**	**31.5±2.5a,d**	**10.5±0.64c**	**52.75±3.03c**
D+400P	**Means±SE**	**40.33±2.6a**	**5.67±0.88a,d**	**19±2.64b,c**	**15.67±0.33a**	**77.67±1.85a**
D+200 F.v.	**Means±SE**	**46±3.78a,c**	**11.33±0.66c,d**	**25.33±4.05a,c,e**	**9.33±0.33c**	**46.33±1.85c**
D+400 F.v.	**Means±SE**	**59.5±3.92d**	**9.75±2.05c,d**	**35.75±5.28d**	**14±0.91a**	**70±4.45a**
D+ (200P+200 F.v.)	**Means±SE**	**44.67±0.33a**	**13.33±0.33c**	**21.67±0.66b,c**	**9.67±0.66c**	**47.67±3.71c**
D+ (400P+400 F.v.)	**Means±SE**	**39±5a**	**13.33±2.4c**	**15.67±2.33b,e**	**10±0.57c**	**50.67±2.96c**
F ratio		**9.27**	**7.54**	**5.71**	**96.41**	**93.82**
Probability		***	***	***	***	***

Mean with dissimilar superscript letter are significantly different at ($P<0.05$)
($p<0.05$) =* ($p<0.01$) =** ($p<0.001$) =***

Discussion:-

These studies suggested that the propolis and *Foeniculum vulgare* have beneficial effects on lipid profiles in alloxan induced diabetes rats. Regarding to the results of total cholesterol, LDL-C, VLDL, and TG. It shows a significant decrease. And significant increase in HDL-C ($p<0.05$) in Control (Non diabetics), Diabetics + treated with Propolis 200mg, Diabetics + treated with Propolis 400mg, Diabetics + treated with *F. vulgare* 200mg, Diabetics + treated with *F. vulgare* 400mg Diabetics + treated with (*F. vulgare* 200mg+Propolis 200mg) and Diabetics + treated with (*F. vulgare* 400mg+Propolis 400mg), when compared with Positive control (Diabetics) may be due to defect in liver metabolism, liver plays a critical role in discharging cholesterol via bile secretion this results agreement with **(El-Sayed *et al.*, 2009) and (Al-Hariri, 2011)** reported that propolis of Egyptian origin have antihyperlipidemic effect by significantly ameliorating the elevated level of LDL-C, TC, TG, and decreased HDL-C in diabetes. **(Raghuveer *et al.*, 2013)** reported that the antidiabetic activity and Anti hyperlipidemic activity of *Foeniculam vulgare*.

Conclusion:-

Propolis and *Foeniculum Vulgare* possesses hypolipidemic activities

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