***Ministry of Higher Education and***

 ***scientific Research***

**Diyala University**

**College of Veterinary Medicine**

***Graduation Research Entitled***

***The effect of polium on Triglyceride and cholesterol and Total protein in experimental animal (rabbits)***

***Submitted by the student***

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***To the Board of the factually of Veterinary Medicine at the University of Diyala , Which is part of the requirements of the bachelor's degree in Veterinary Medicine and surgery***

**Under the supervision of**

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***Dedication***

***This research paper is dedicate ……***

***Who spent their lives for me … My Father and my Mother***

***And all whom helped me to achieve this research***

***And special thanks to My research's supervised***

***Ass. Pro. Ahmed Jassim Mohammad Reza Albayati***

***Special thank***

***I would like to address special thanks to my advisor (Ass.pro.Ahmed jassim mohammad***

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 ***providing me with moral support and very useful materials.***

 ***Abstracts***

***In*** ***these study used 20 rabbits divided in to 5 group each group contain 5rabbits the effect of polium on total protein and triglyceride and cholesterol in the pigeon in different concentration when we comparative to control . it is decrease the level of total protein (T.P)and triglyceride (T.G)and cholesterol (C.h) these depend on concentration of when increased the concentration of polium it is decrease the (T.P,T.G,C.h) it is act antioxidant and effect on LDL and LDH.so The poleum act effect of level in the blood.***

 ***Introduction***

Liver is the major organ of the body that has an important effect on carbohydrates and lipid metabolism (1). In the presence of insulin, glucose is used but lipids and proteins are stored in the body (2). In diabetes mellitus, insulin deficiency leads to failure of glucose consumption, consequently results in breakdown of lipids and proteins (3). In traditional medicaments, Teucrium polium is used as analgesic, anti-spasmodic and hypolipidemic agent (4-6). Visceral analgesic effects of T. polium extract compete considerably with those of indomethacin and hyoscine (4). Use of T. polium in saccharomyces's culture media in vitro led to decrease in fatty acids and acts as anti-fungal, anti-bacterial and anti-inflammatory agent, and blocks the peroxidation of erythrocytes (7- 9). There is an agreement for hepatotoxicity of T. polium administration (10). Administration of 150 mg/kg Teuceium polium extract was showed to act as an anti-ulcer agent (11). Intravenous infusion and i.p. injection of plant extract after 4 and 24 hours led to decrease of blood sugar in rats (12). Oral and i.p. administration of dried aerial parts and bloom extract of T. polium decreased appetite, water and food consumption and consequently body weight in rats (13). The side effect of T. polium extracts were reported in diabetic patients who used it as an anti-diabetic agent (14, 15). Oral

administration of alcoholic T. polium extract showed no changes in fasting and postprandial blood sugar in diabetic patient (16). Zal et al. (17( reported that the administration of T. polium boiling extract had an anti-diabetic effect on diabetic rats )17(. With considering the controversial reports of the above studies, the prime aim of this study was to identify the the effect of T. polium aqueous extract on blood glucose, Liver enzymes linked to liver dysfunction and serum lipid in streptozotocin diabetic male rats .

***literature review***

An individual with insulin resistance who has relative rather than absolute insulin deficiency afflicted type 2 diabetes. Insulin deficiency leads to failure of glucose consumption in diabetes mellitus (DM) and consequently results in breakdown of lipids and proteins. DM induces a group of syndromes characterized by insulin resistance and hyperglycemia – altered metabolism of lipids, carbohydrates and proteins – and an increased risk of cardiovascular disease complications (18). High carbohydrate diets and excessive total calories are associated with much higher Triglyceride content in serum, liver and muscle (19). Some evidence suggests that an excess accumulation of hepatic and skeletal muscle lipid is associated with insulin resistance and type 2 diabetes mellitus in human (19,20) and animal models (21, 22). Dietary sugar or sucrose content is the major focus of carbohydrate-based dietary guidelines. Sucrose consumption, especially its fructose component produces pre-diabetic status. After the absorption in the gastrointestinal tract, fructose is transported via the portal circulation to the liver, where it enters hepatocytes via the glucose transporter GLUT5-independently of insulin (23, 24). Phosphofructokinase, a hepatic enzyme that governs glycolysis in liver, negatively regulates glucose breakdown while fructose can evade this rate-limiting control mechanism and is metabolized into glycerol-3-phosphate and acetyl-coenzyme A. These two intermediate metabolites are then used as substrates for glyceride synthesis, contributing to very low-density lipoprotein triglyceride production in the liver (25, 26). The exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and triglyceride accumulation, which in-turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance (27).

Many herbal formulations have been recommended for the treatment of diabetes as an alternative for the currently available therapeutic options like oral hypoglycemic agents and insulin therapy (28). In the field of alternative medicine, *Teucrium Polium L.* (*Labiatea*) is known to have hypoglycemic effects and it is widely suggested to the diabetic patients in Iran and throughout the world. *T. polium* (Calpoureh) is a member of Labiatea family which are well-known to possess antibacterial, anti-inflammatory (29,30), antidiabetic and hypoglycemic (17-20), antihypertensive (1) and antilipidemic (21) activities. Unfortunately, most aqueous and organic extracts of the plant are hepatotoxic (22). There are few reports relating to the hepatoprotective and antioxidant effect of *T. polium* ethyl acetate extract (23, 24). Both properties are very useful in controlling diabetes. To our knowledge, there are a few reports about the hypotriglyceridemic effect of TP-EAE on carbohydrate-induced hypertriglyceridemia, so this study was conducted to investigate the effect of TP-EAE on serum, muscle and liver lipid profiles, also insulin resistance in pre-diabetic rats.

*Plant material*

*Teucrium polium* L. (Lamiaceae) samples were collected from Izeh local area, Khuzestan, Iran. The dried leaves of *Teucrium polium* were authenticated by Faculty of Agriculture, Shahid Chamran University of Ahwaz, Iran.

*Preparation of plant extract*

Fresh leaves of *Teucrium polium* were separated, cleaned and dried at room temperature. The dried powdered plant material (300 g) was extracted by continuous mixing with ethanol (70% and 80%), at room temperature for 24 h. After filtration, ethanol was evaporated until only water remained. Water phase was subsequently extracted with ethyl acetate, then filtered and concentrated under the vacuum condition up to a concentration of 1 g /1 mL of extract (23, 25).

*Experimental animals*

Healthy adult male Wistar rats weighting 180 20 ± g were purchased from Physiology Institute of Ahvaz (Iran). Animals were housed in cages under the conditions of controlled temperature (25°C), relative humidity of 65 ± 10% and a 12 h artificial light period for 10 weeks and had free access to water and standard pellet diet. The experiments were carried out after the approval

***Material***

***1.poleum***

***2.starch***

***3.kaging***

***4.test tube with anti coagulant(EDTA)***

***5.spectophatometer***

***6.kiten***

***7.Exeperimental animal (rabbits)***

***method***

***1.taken 20 rabbits and divided in to 5 group each group contain 4 pigeon.***

***2. group 1 gives 25% from poleum and starch (mix 25gm of poleum and 75starch)in capsules given to rabbits orally .***

***3. group 2 give50% from poleum and starch (mix 50gm of poleum of and 50gm starch )in capsules given to rabbits orally.***

***4. group 3 give 75%from poleum and starch (mix 75gm of poleum and 25gm starch )in capsules given to rabbits orally.***

***5.group 4 give 100%from poleum(mix 100gm of poleum)in capsules given to rabbits orally.***

***6.group 5 this group are control which give starch only not mix poleum.***

***7. in 1day give capsules given to rabbits orally e***

***for about 1month.***

***8.after 1month collect of blood from each group of rabbits and that make laboratory analysis***

***of cholesterol and triglyceride and total protein ratio of rabbits (that give poleum and starch ) and ratio comparative with ratio of cholesterol and triglyceride and total protein of control rabbits.***

***Determination of cholesterol***

***principle of the method:***

***the cholesterol present in the sample originate a coloured complex***

***According to following***

$$cholesterol+H2o \rightarrow cholesterol+fatty acid$$

$$cholesterol+O2\rightarrow cholesterol+H2o2$$

***2H2o2***$+phenol+minophazone\rightarrow Quinonimine+4H2o$

***The intensity of the color is proportional to the cholesterol concentration in the sample***

***Procedure***

***1.assay condition***

***Wavelength…………………………….505 nm (500-550)***

***Cuvette……………………………….1cm light path***

$$Temperature..……….…37℃\15-25℃$$

***2. Adjust the instrument to zero with distal water***

***3.pipette in to a cuvette***

|  |  |  |  |
| --- | --- | --- | --- |
| ***sample*** | ***stander*** | ***Blank*** |  |
| ***1.0*** | ***1.0*** | ***1.0*** | ***WR(ML)*** |
| ***10*** | ***…….*** | ***…….*** | ***Standard (uL)*** |
| ***10*** | ***……*** | ***…...*** | ***Sample(uL)*** |

***4.mix and incubate for 5 min at 37c or 10 min at room temperature.***

***5.Read the absorbance (A) of sample and stander. Against the Blank. The coloure is stablefor at least 60 min.***

***Calculation:***

***(A)sample\(A)stander \*200(standard conc.)=Mg\dl cholesterol in sample***

***Conversion factor : mg \dl\*0.0258=mmol\L***

***Determination of total protein***

***Protein give an intensive voilat-bule complex with copper in an alkaline medium. Iodide is include as antioxidant. The intensity of the color formed is proportional to total proteion concentration in the sample.***

***Procedure***

 ***1.Assay condition :***

***Wavelength……………..540 (530-550)nm***

***Cuvette……………………..1cm.light path***

***Temperature ……………….37C\15-25C***

***2. Adjust the instrument to zero with distalled water.***

***3.pipette into a cuvette.***

|  |  |  |  |
| --- | --- | --- | --- |
| ***sample*** | ***stander*** | ***Blank*** |  |
| ***10*** | ***10*** | ***10*** | ***WR (ML)*** |
| ***…..*** | ***25*** | ***……*** | ***Standard (ul)*** |
| ***25*** | ***……*** | ***……*** | ***Sample (uL)*** |

***4.mix and incubation 5 min at 37C or 10 min at room temperature .***

***5. Read the absorbance (A) of the sample and standard against the blank . the coloure is stable at least 30 min.***

***Calculation:***

***(A)sample\(A) standard\*(standard conc.) =g\dl of protein in the sample***

***Determination of triglyceride :***

***Principle :***

***The triglycerides are enzymatically hydrolyzed to glycerol according to the following reaction:***

$$Triglycerid\rightarrow glycerol+fatty acids$$

$$Glycerol+ATP\rightarrow glycerol-3-phosophat+ADP$$

$$Glycerid-3-p\rightarrow Dihydroxyactone-p+H2o2$$

$$H2o2+4-aminophenazone+p-chlorophenol\rightarrow H2o+Quinoimine$$

***Procedure:***

***Wavelength…………………….505nm (490-550)***

***Temperature ………………….37C(25C)***

***Cuvette……………………….1cm light path***

|  |  |  |  |
| --- | --- | --- | --- |
| ***sample*** | ***stander*** | ***Blank*** |  |
| ***……..*** | ***10ul*** | ***……..*** | ***stander*** |
| ***10ul*** | ***…...*** | ***…..*** | ***sample*** |
| ***1Ml*** | ***1Ml*** | ***1Ml*** | ***Working reagent*** |

***Mix and incubation 5 min .at 37C or 10 min .at 25C.the coloure is stable for 30 min .***

***Calculation :***

***Triglycerides conc.= O.D sample\ O. D standard***

***Result :***

 ***In these study the effect of poleum in the Different concentration on total protein and triglyceride and cholesterol . in the 25% there is effect on triglyceride but on the cholesterol and total not effected and in the concentration of 50% of polium effected and in the concentration of 75%there is no effect in the concentration of 100% there is effect of poleum.***

|  |  |  |
| --- | --- | --- |
|  Conc.Parameter | Control | Treatment |
| control | 10% | 25% | 50% | 75% |
| triglyceride | Mean= 19-Es = + 4.1 | Mean= 12.7-Es = + 1.8 | Mean= 16.5-Es = + 2- 5 | Mean= 5.2-Es = + 18.2 | Mean= 8.7-Es = + 2.1 |
| Total protein | Mean= 8.4-Es = + 2.7 | Mean= 14.2-Es = + 3.8 | Mean= 6.1-Es = + 6.1 | Mean= 7.2-Es = + 7.2 | Mean= 7.2-Es = + 1.4 |
| Cholesterol | Mean= 23.9-Es = + 11.9 | Mean= 76.6-Es = + 13 | Mean= 40.4-Es = + 9.4 | Mean= 37.2-Es = + 17.3 | Mean= 29.8-Es = + 4.8 |

20

Ch 15

T.G 10

T.p 5

**concentration**

**zero**

(Diagram1) the control with concentration

20

Ch 15

T.G 10

T.p 5

**10%**

(Diagram2) the concentration of 25% of polium .

20

Ch 15

T.G 10

T.p 5

**25**

( Diagram 3 ) concentration of 50% of polium.

20

Ch 15

T.G 10

T.p 5

(Diagram4)the concentration of 75 % of poleum.

20

Ch 15

T.G 10

T.p 5

**concentration**

(Diagram5) the concentration of 100% of poleum .

***Discussion***

 ***It is well known that a successful for treatment of dyslipidemia is primary prevention of postprandial hyperlipidemia by aggressive delaying fat digestion and absorption ( 1 , 2 ) . Previously , it has shown that oligomer procyaniding containing . it was found that degree of polymerization of oligomer procyaniding was an important factor to in crease potency on pancreatic lipase inhibition (3 ) . In the study the effect of poleum at 75% on then triglyceride is sigil these Agree with ( 4 ) but on the total protein is not affect ( 5 ) . on the control it is affect but not significant (6 ) . these compares with control (rabbit) . the Concentration of 25% the effect of poleum on triglyceride is significant ( 7 ) . on the total protein and cholesterol is significant ( 8 ) . on the conciliation of 50 % of poleum there is no effect on the triglyceride and total protein and cholesterol on the concentration of 75% of poleum there is effect of triglyceride and the affect is signification and the ( 9 ) on total protein and cholesterol is significant ( 10 ). Finding showed that acute administration of poleum markedly suppressed the elevation of serum triglyceride and cholesterol and total protein in high concentration of poleum of 75% (11 ,12 ,13 ). The poleum reduces plasma lipid profiles and prevents a high 100% . fat diet mduced obesity in hamster and related metabolic (14 ) . the supplemented with poleum inhibits progression of Atherosderosis in cholesterol in rabbits ( 15 ) . the mechanism of action in related to prevention of low . density lipoprotein ( LDL) oxidation in the arterial well diet( 16 ) .***

 ***We suggest that large term and high concentration supplementation of poleum reduced plasma liquid total protein and cholesterol and triglyceride and diabetes from this point of view , an intake of poleum may be a feasible therapeutic strategy for prevention and treatment of patient with hyperlipidemia and obesity and diabetes .***

**Conclusion**

The *Teucrium polium* ethyl acetate extract modulates the serum, liver and muscle TG, and improves the insulin resistance in the experimental rat fed by sucrose-rich diet which may be useful in preventing or early treatment of diabetic disorders. However, further studies are needed to determine possible mechanisms of action, but these effects may be attributed in part to hypolipidemic effect of *T. polium* flavonoids, otherwise, the hepatoprotective and antioxidant activity of TP-EAE may improve the function of liver and reverse the harmful sucrose effects.

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