Effect of phenolic compounds Extractions of Green tea (*Camellia sinesis L*) On the glutathione –S- Transferase Alloxan Experimental Induced-Diabetic Rabbit.

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Abstract

Diabetes Mellitus is considered as a member of oxidative stress syndrome. It is associated with an imbalance between types of free radicals and scavenger's system. This study showed the effect of the polyphenol compounds extracted from green tea (Camellia Sinesis L.) on alloxan induced diabetic rabbits to determine their role in treatment of diabetes mellitus, their effect on enzymatic antioxidant and to know the histological effects on the diabetic kidney. The rabbits group was divided into four groups, each group consist of 7 rabbits. The first group was healthy rabbits (normal group) as compared With second group .The second group diabetic without treatment as control group with the last three groups. The third group diabetic treated with extract of green tea 100mg/kg of body weight as a single daily dose. The fourth group diabetic treated with extract of green tea 200mg/Kg of body weight as a single daily dose. After treatment for twenty week blood and tissue samples were taken for analysis and the result were as follow : There was a significant decrease in GSTS. When polyphenol extract of green tea was used with dose rate 100mg /kg of body weight (fourth group) gave a significant increase ($p<0.05$) in all parameters as compared with diabetic group (without treatment). The present results revealed that alloxan was effectively induced diabetes by partial destruction of b-cells of pancreas which lead to elevation of blood glucose level. As a consequence of hyperglycemia the abnormal effect was obvious in certain tissues in the body which attributed to the effect of diabetes .Histological investigations shows that all the lesions in the kidney that result from diabetes such as hypertrophy, degeneration and hylanosis of the glomeruli.

تأثير مركبات الفينولك المستخلصة من بذور العنب على أنزيم كلوتاثيون ترانزفيريز- ايس في الارانب المستحدثة مرض السكري بمادة الالوكسان

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المستخلص

يعد مرض السكري من العناصر المتلازمة لضغط الموكسدات ذات الصلة بالتوازن بين أنواع الأوكسجين الفعال ونظام الكاسحات,استهدف هذا البحث دراسة تأثير المركبات الفينولية المستخلصة من الشاي الاخضر camellia sinensis L. في الارانب المحدث بها السكري بواسطة الالوكسان لمعرفة علاج داء السكروتأثيرهما على الأنزيم الكلوتاثايون ولمعرفة التأثيرات النسيجية التي تطرأعلى الكلية.شملت هذه الدراسة الدراسة على ثمانية وعشرون من الأرانب وبعمر (٦-١٠) شهرا وقسمت إلى مجاميع,كل مجموعة تضم ٧ من الأرانب.المجموعة الأولى: مجموعه أرانب أصحاء(مجموعه سيطرة)للمجموعة الثانية . المجموعه الثانيه :أحدث بها سكري وبقيت بدون علاج (مجموعه سيطر)للمجاميع الثلاث الأخيرة .المجموعه الثالثه و الرابعة أحدث بها سكري وعولجت بخلاصة الشاي الأخضر و بتركيز ١٠٠ ملغم/كغم و٢٠٠ ملغم/كغم على التوالي من وزن

الجسم جرعة مفردة يومية .استمرت فترة العلاج لمدة ٢٠ أسبوع وبعد الانتهاء من العلاج تم اخذ العينات الدموية والنسيجية لفحصها وتحليلها وتم الحصول على النتائج الاتية. نقصان معنوي في الأنزيم الكلوتاثايون حيث أحدثت الخلاصة الفينولية للشاي الاخضر ارتفاعا ملموسا إحصائيا ($p<0.05$) عندما استخدمت بتركيز ١٠٠و٢٠٠ ملغم من وزن الجسم مقارنة بمجموعة السيطرة.اشارت النتائج الى ان الالوكسان يحدث مرض السكر, ويرفع مستوى سكر الدم من خلال تحطيمه الجزئي لخلايا بيتا في البنكرياس , ونتيجة لارتفاع السكر تظهر تغيرات نسيجية في الكلية المدروسة والتي عزيت الى الاصابة بمرض السكر, والتي تتضمن وعلى سبيل المثال التضخم و الانحلال والتحول الزجاجي للكبيبات في الكلية.

Introduction

Green tea is natural dried leaves of the tea plant, (camellia sinensis). The active constituents of green tea contain volatile oils ,vitamins, minerals , caffeine, and polyphenols particularly the Catechins called epigallocatechin gallate (EGCG).Many in vitro studies show that the flavonoids present in tea have strong antioxidant and metal-chelating properties and may therefore protect cells and tissues against free oxygen radicals. A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases. Reactive oxygen and nitrogen species are formed in the human body and endogenous antioxidant defenses are not always sufficient to counteract them completely [1]. Improper absorption of blood sugar leads excess concentrations that must be released through urine if this continues for long periods of time it can lead to a number of more serious illnesses The body cant absorb the starch molecule as a whole- it has to be broken this part by special enzyme called amylase does just that, acting like a pair of scissors to cut each part loose from the string these single part are then able to be absorbed into the blood stream but green tea polyphenol have been found to be potent inhibitors of amylase [2]. It's as if green tea dulls the enzymes cutting and rusts its scissors –like effect until it's almost worthless. In laboratory tests , the amount of polyphenol in just one cup of green tea was found to inhibit 87% of amylase activity and if less sugar gets into the bloodstream , blood glucose levels will automatically be lowered[9]. The polyphenol groups of green tea Catechins have been shown to lower blood sugars [3]. Glutathione-s-transferase (G-ST) are recognized as important catalysts in the biotransformation of xenobiotics, including drugs as well as environmental pollutants. Multiple forms exist, and numerous transferase from mammalian tissues, insects, and plants have been isolated and characterized enzymatic properties, reactions with antibodies, and structural characteristics have been used for classification of the glutathione transferase[4].

Materials and Methods

Twenty eight (28) healthy local adult rabbits of age ranged (6-10 months) and their body weight ranged (1.5 – 2.5) kg obtained from local market of Baghdad city. Animals were divided into 4 groups each contais 7 animals, as follows:-Group 1: Healthy rabbits ,Group 2: Diabetic control rabbits . Group 3, and 4: Diabetic rabbits treated with extract of green tea as a single daily dose.100,and200mg/kg body weight of extraction dissolve in 1cc DW orally for 20 weeks respectively.

Extraction of phenolic Compound

The leaf of green tea and grape seed (yellow seed) were dried and powdered, according to Gayon method [5]. 200 gram of plant powder was

weight and added to 800ml of 2% acetic acid and extracted, the mixture was left for 24 hours in an incubator at 50C°, then filtered through filter paper to remove all the residual materials. The clear extracted solution treated with the same volumes of n- propanol, and then saturated with Nacl. The upper layer was separating by funnel, then dried at 45C° using an incubator.

Pilot study: The dose used in this study was estimated according to the result of pilot study.

Induction of diabetes mellitus

Diabetes was induced in rabbits by injection of alloxan tetra hydrate at a dose of 180 mg /kg body weight IV In marginal ear vein [7]. Soon the animal were injected with 10 ml of 20% glucose solution S/C.Glucose solution 10% was given for 24 hours instead of the tap water in order to reduce alloxan hypoglycemic shock .

Biochemical analysis:

Estimation of GST

Determination of serum glutathione (8). 5,5-Dithiobis (2-nitrobenzolic acid) (DTNB)is a disulfide chromogen that is readily reduced by sulfhdryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromagen is measured at 4/2 nm and is directly proportional to the GSH concentration.

Tissue preparation for light microscopy

The animals were survived for their end period and then sacrified using high dose inhalation of chloroform inside glass box .The animals were dissected, ,the abdomen was opened by a longitudinal incision, and a few drops of the fixative were put on the kidneys before they were dissected out within a few seconds. Then dissected kidneys and immersed in 10%formalin for 24h for fixation and histological tissue processing purposes . After fixation each kidney sample were cut with a blade to small piece . The specimens washed in running tap water, dehydrated through graded ethyl alcohol,50,70,90,100,half hour for each, and cleared by xylol. Then, embedded in melted paraffin inside paraffin bath at 64 C for half hour and blocked in paraffin wax. All sections then immersed in xylol for 10 minutes,rehydrated through graded alcohol 5-10 minutes for each,and finally imbedded incide,water for 3 minutes . Sections were stained using haematoxylin and eosin. Then placed in graded alcohol,50,70,90,100.

Results

The effect of green tea intake on GSTS *level in alloxan diabetic rabbit*

Table (١) showed obtained that the GSTS levels is significantly increased in alloxan diabetic rabbits P<0.05 (4.31± 1.8) µ/L compared with (2.70±1.7) µmol/L in control rabbit. After treatment with 100 mg of green tea a significant decreased in GSTS level (3.01±0.38) µ/l. was observed compared with control group which indicated a positive correlation effect of green tea intake. The level of GSTS was significantly reduced P<0.05 (2.98±1.8) µmol/L in alloxan diabetic rabbits receiving 200mg of green tea compared with the level of control group as shown in table(1).

Table (1):- Means and standard deviations of GSTS levels in healthy and alloxan diabetic rabbits after twenty weeks of daily administration of (100mg/kg, and 200mg/kg), of green tea.

Group	GSTS (µ/L)
Healthy rabbits	2.70±1.7
Alloxan diabetic rabbits	4.31± 1.8
Alloxan diabetic rabbits after green tea 100mg intake	3.01±0.38
Alloxan diabetic rabbits after green tea 200mg intake	2.98±1.8

*: Significant compared with diabetic group

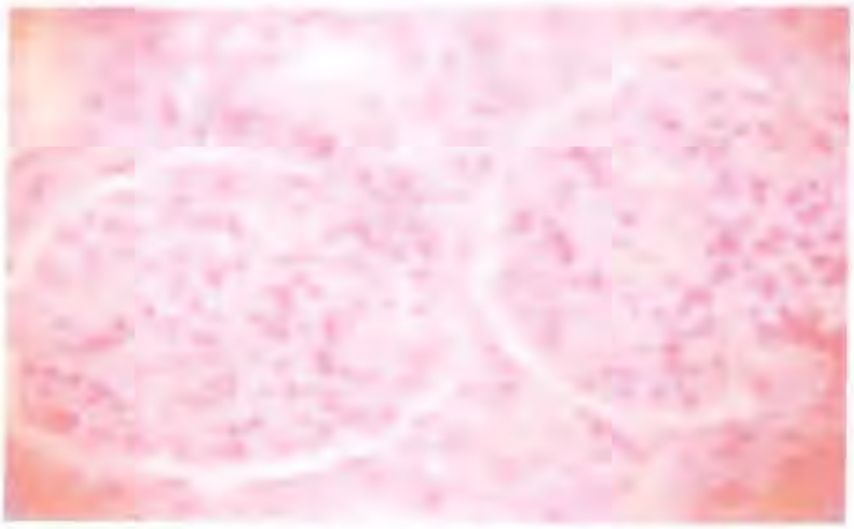

Fig.(2):-Hhypertrophy of glomeruli in diabetic group. H&E.40X.

Fig.(3):-Glomeruli appear nearly normal , proximal convoluted tubules appear nearly normal, distal convoluted tubules appear nearly normal in green tea group after treatment. H&E.40X.

Discussion

As shown in table (2) a significant decrease in the levels of GSTS were obtained in alloxan induce diabetic rabbits receiving polyphenol compound daily for 20 week with before treatment.GSTS is an antioxidant enzyme that catalyzes the reaction between reduced glutathione (GSH), drugs ,xenobiotic and other toxic compounds, rendering them more water soluble and finally exerted from the body [9]. Some chemical compounds, which augments oxygen products have generated toxic effect such as many drugs free radical. The radical reduces the molecule oxygen immediately, the superoxide anion(O2) comes into existence and , following that other reactive oxygen species (ROS) are generated [10,11].

Microscopic investigation

In diabetes mellitus frequently damage the kidney, leading to impaired excretory and homeostatic function. Decreased blood flow through the glomerulr capillary system because of the thickening of the arteriole and arteriolar walls , and the consequent reduction in the Lumina of this vessels, produces chronic ischemia of the tubular system and reduces glomerular filtration if prolonged, this lead to disuse shrinkage of the compounds of the glomerulus (glomerular hyalinization) and atrophy of the tubules. When this changes affected most of the glomeruli and their associated tubular system all of the functions of the kidney are impaired. A good glycemic control may help in the prevention of glomerular hypertrophy and preventing many lesions that accompanied diabetic nephropathy, this was in according with Ballard *et al* [12], and Vasquez *et al*[13].Glomerular hypertrophy and mesangial expansion which are typical changes in diabetic nephropathy[14,15]. Factors contributing to glomerular enlargement in diabetic nephropathy include increased intraglomerular pressure, growth of glomerular cells,

and accumulation of extracellular matrix [15]. The present study shows that phenolic compounds especially in 200mg of green tea, have most positive results in preventing many lesions that accompanied diabetic nephropathy.

References

1- Moyers S.B., Kumar N.B., Green tea polyphenols and cancer chemoprevention: _Multiples mechanism and endpoints for phase II trials, Nutr., 2004. May, 62(5):204-11.

2- Al- Kassab A., Effect of aqueous extract of Pistaca altntica Leaves on serum glucose level, M.Sc. Thesis., College of Science, University of Mosul, 1999.

3-Day C., and Bailey C.J., Hypoglycemic agents from traditional plants treatment for diabetes, In:Industrial Biotech., 1988b, 8:5-8.

4-Mannervik,B. and Banielson V. Glutathione Transferase S-structure and catalytic activitycrit. Rev. Biochem. ; 1988; 23(3): 283-337.

5-Gayon, T.A. Plant phenolic. Olive and Boyed, Edinboura.1972.pp254

6- Subra, A. Push, P. and Rajaseck, K. Effect of Artemisia pallens wall. On blood glucose levels in normal and alloxan- induced diabetic rats.J.Ethno. 1996,50: 13-17.

7-Burtis, C. A., And Ashwood, E.R. Tietz Textbook of Clinical Chemistry, 3rd ed., W.B. Saunders Company, Tokyo, 1999, PP.:1034-1054.

8-Habig,W.,Pabst,M., and JakobyW.,Glutathione S-Transferase, the first Enzymatic Step in Mercapturic Acid formation .J-Biol. Chem.1974;249:7130-7139.

9-Metabolic control and disease W.B. Saunders and company, Philadelphia, London , Tornoto, 1980: 276-371.

10-Coughlin S.,and Itall I., Genetic in Medicin:Glutathione S-Transferase polymorphisms and Risk of Ovarian cancer Atlanta.2002;4(4);250-257.

11-Raza H., Prabu, S., Robin,M.and Avadhani N., Elevated Mtichoudria cytochromic p450 2EI and Glutathione S-Transferase in streptozotocin – Induced Diabetic Rats .Diabetis ,2004;53:185-194.

12-Ballard D.J., and Palumbo D.J.:Epidemiology of persistent protcinuria in type 11 diabetes mellitus .Diabetes .1988;37:405-421.

13-Vasquez B., Flock E.V.,Savage P.J., and Bennett P.H.: Sustained reduction of proteinuria in type 11 diabetes mellitus following diabetic induced reduction of hyperglycemia .Diabetolgia.1984;26:127-133.

14-USRDS. ADR. Bethesda, MD: NIH, NIDDK, April 1999, Appendix, table A-6.

15-Eggers, P.W. Personal communication. Health Care Financing Adminstratin.1999.

16-USRDS. ADR. Bethesda, MD: NIH, NIDDK, April 1999, Appendix, table A-1.