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Biological studies on green coffee beans extracts and its relationship with obesity and diabetes mellitus diseases

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Abstract: This study was conducted to investigate the effect of green coffee beans consumption on obesity and diabetes mellitus diseases. Twenty five Albino rats weighting (190 ±20 g) were divided into 5 equal groups, one was kept as a control-Ve group, While the other 4 groups were injected by alloxan after induction of obesity. Obese diabetic rats were fed orally with green coffee extract (10-20%) by the dose 4mg.day^{-1}. Serum liver enzymes activities (ALT, AST and ALP), kidney function parameters (creatinine and urea levels), serum lipid profiles (TG, LDL-c and HDL-c), serum lipid oxidation parameter (TBARS) and glucose level were examined. The obtained results revealed that green coffee beans extract, contains several classes of phytochemicals with other compounds, are able to prevent or inhibit obese and diabetes complications through liver serum enzymes-lowering activity, kidney function parameters concentration lowering, enhancing the serum lipid profile and decreasing rate on the formation of TBARS in serum. In conclusion, data of the present study recommended green coffee extract by a concentration 4 mg.day^{-1} %, amount to be included in our daily diets, drinks and food supplementation

Key words: Serum glucose, phytochemicals, liver functions, kidney functions.

Introduction

Obesity, a chronic disequilibrium between food consumption and energy expenditure, continues to be a major health problem in developed

and developing countries. Obesity is the fifth leading risk for global death. At least 2.8 million adults die each year as a result of being overweight or obesity (WHO, 2008). The world health organization predicted that there will be 2.3billion over weight adults in the world by 2015, and more than 700 million of them will be obese (WHO, 2011). Obesity, the accumulation of excess body fat, results when energy intake exceeds energy expenditure so weight reduction is achieved with negative energy balance to reduce body weight by decreasing caloric intake and /or increasing energy expenditure (Pamela *et al*., 2005). Treatment of overweight and obesity continues to involve: A healthful lifestyle that includes increased physical activity, reduced total energy intake and behavior therapy is the foundation of a comprehensive weight management program (Onakpoya *et al*., 2011).

The prevalence of obesity and type 2 diabetes is rapidly increasing around the world and its growth has become a major challenge for health care professionals to combat (Hossain *et al*., 2007 and young, 2010). The incidence of obesity and related metabolic disease is increasing globally With the high cost of prescription weight loss drugs and the fear of side effects, current medical treatments often fail to half the progress of such disturbances, the general public is turning to nutraceutical is the estimated global market for 24 is over 350 billion dollars, as published by market research news. Recently, the bioactive components in foods and functional foods have become popular and been considered as complementary or alternative therapeutic agents to manage and /or treat chronic disease. More over it is illustrated that in addition to standard prescribed therapies a host of complementary and alternative herbs and dietary supplements are widely used to manage obesity and diabetes (Connell, 2001). Therefore a variety of natural products, including crude extract and isolated compounds from plants may be an excellent alternative strategy for developing future effective, safe anti obesity drugs (Park *et al*., 2005, Nakayama *et al*., 2007 and Mayer *et al.*, 2009).

One supplement that has gained considerable popularity in the recent year is green coffee beans extract (GCE). The supplement contains naturally occurring caffeine and chlorogenic acid, a polyphenol antioxidant. Green coffee beans are used because the roasting process of coffee beans reduces the chlorogenic acid levels. Chlorogenic acid is thought to be responsible for pharmacological effects of green coffee

beans (NNCD, 2012). It has been shown to inhibit fat accumulation and reduce weight in animal models and human. In addition GCE is thought to reduce postprandial glucose concentrations. It is also thought to reduce glucose absorption in the intestine. There is also speculation that GCE might alter adipokine levels and body fat distribution (Onkpoya *et al*., 2011).Although many studies have been carried out on the effect of green coffee beans on prevention / treatment of different diseases, the data regarding their effect on diabetes mellitus and obesity still in dearth. Therefore, the present study aims to investigate the effect of green coffee beans consumption on obesity and diabetes mellitus diseases.

Materials and Methods

Materials

Green coffee beans, were purchased from local markets, Cairo, Egypt. All chemicals and solvents used in the analyses were analytical grad. Alloxan, used in diabetes induction, was purchased from Techno-Gene, Chemical Co., El Doki, Egypt. Male white albino rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Components of basal diet were purchased from El-Gomhoria Company, Cairo, Egypt. Corn oil and tallow, used in obesity induction, were obtained from local market, Cairo , Egypt. Kits for aspartate amino transaminase (AST) and alanine amino transaminase (ALT) developed by Diamond Diagnostics, Cairo, Egypt. Alkaline phosphatase (ALP), urea, total cholesterol, triglycerides and high density lipoprotein (HDL) were obtained from Biodiagnostic Company, cairo, Egypt.

Methods

Preparation of green coffee beans samples

Green coffee beans were milled by using a mill (Moulinex, ElAraby Co., Benha, Egypt) to give a powder and were kept in plastic bags in a cool and dry location for using according to Russo, (2001). Green coffee beans samples were prepared with different concentrations (10, 15 and 20 g.100 ml^{-1}) using Microwave oven (ElAraby Co., Benha, Egypt). Grams of grinded green coffee beans were solved in 100 ml of distilled water and extracted at 50 ºC for five min and followed by

filtration according to Rohit Upadhyay *et al*., (2012) with some modifications.

Biological experimental

Twenty five white male albino rats, Sprague Dawley strain, 2 week age, weighting (190 ±20 g) were used. Rats were kept in cylindrical wire cages with wire bottoms. The diet was introduction in special food cups to avoid scattering of food. Also water was provided to the rats by glass tube projection through the wire cage. Food and water provided ad-labium and checked daily.

Experimental design

All biological experimental were done at animal research laboratory, Faculty of Home Economics, Minoufiya University, Egypt. Rats (n=25) were housed individually in wire cages in a room maintained at 25±2 ºC and kept under normal healthy conditions. All rats were fed on basal diet (casein 12.5%, corn oil 10%, Minerals mixture 4%, Vitamin mixture 1%, fiber 5%, DL-methionine 0.3, Choline chloride 0.2 and completed to 100 % by corn starch according to Ain (1993) for one week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups:

Group (1), negative control group (5 rats): in this group, rats were kept on basal diet and tap water.

Group (2): Obese diabetic rats groups (20 rats): In this group, rats were kept for four weeks on high fat diet to induce obesity. High fat diet prepared from fine ingredients per 100 g according to Negm, (2002). The diet had the following composition: Fat, 20% (tallow 10% + corn oil 10%); casein (protein) content, 12.5%; salts mixture, 3.5%; vitamins mixture, 1.0%; fiber, 5%; Dl-methianine, 0.3%; choline chloride, 0.2 %; and corn starch up to 100g according to AIN, (1993). After induction of obesity the obese rats were injected by alloxan 150 mg /kg body weight according to the methods described by Desai and Bhide, (1985). One week after the injection of alloxan, fasting blood samples were obtained to estimate fasting serum glucose. Rats having fast serum glucose more than 160 mg/dl were considered diabetics (NDDG, 1994). All the obese-diabetic rats were classified into 5 groups (5 rats per each) as follows:

- Sub group 1 (-Ve): Negative control group, in which normal rats fed on basal diet all experiment period (28 days) .
- Sub group 2 (+Ve): Positive control group, in which obese diabetic rats fed on high fat diet all experiment period.
- Sub group 3: In which, obese diabetic rats fed a high fat diet + 4ml of GCB 10% orally /day.
- Sub group 4: In which, obese diabetic rats fed a high fat diet + 4ml of GCB 15% orally /day.
- Sub group 5: In which, obese diabetic rats fed a high fat diet + 4ml of GCB 20% orally /day.

Blood sampling

In all experimental groups, blood samples were collected after 12 hours fasting at the end of each experiment, in which the rats were sacrificed under ether anesthesia. Blood samples were received from portal vein into clean dry centrifuge tubes in which blood samples left to clot in it at room temperature then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirated and transferred into clean cuvette tubes. All whole blood and serum samples were stored frozen at -20 ºC for biochemical analysis (Malhotra, 2003).

Analytical methods

Determination of liver functions

Determination of ALP and AST were carried out by kinetic method according to Rec, (1972) and ALP according to Reitman and Frankel, (1957). Creatinine was carried out by colorimetric method according to Henry, (1974). Urea was achieved by enzymatic method according to Patton, 1977). Triglycerides was determined by enzymatic colorimetric test according to Trinder, (1969). HDL-cholesterol was carried out according to Rhichmond, (1973). Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were determined according to the Friedewald *et al*., (1972) equations as follow:

VLDL-c (mg/dl) =Triglycerides/ 5.

LDL-c (mg/dl)= total cholesterol (TC) - (HDL+VLDL).

Glucose was determined by enzymatic colorimetric method according to Trinder, (1969).

Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigma stat, statistical software, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple rang test at $P < 0.05$ to indicate significance between different groups (Snedecor and Cochran, 1967).

Results

Liver Functions

Feeding of the rats orally with green coffee extract (10-20%) by the dose 4mg.day^{-1} prevented the rise of mean serum AST, ALT and ALP activities. The rate of preventative was increased with the increasing of the extracts concentration. Such as shown in figures (1), the rate of decreasing in the liver enzymatic activities were recorded 54.47,47.31and 16.54 % (For AST); 92.83,87.63 and 45.80% (for ALT) and 58.56,51.60 and 18.95 % (for ALP) with the rat fed by 4mg.day^{-1} of 10, 15 and 20% extracts of green coffee, respectively.

Kidney Functions

Feeding of the rats orally with green coffee extract (10-20%) by the dose 4mg. day-1 prevented the rise of mean serum urea and creatinine concentrations. The rate of preventative was increased with the increasing of the extracts concentration. Such as shown in figure (2), the rate of decreasing in the kidney function parameters were recorded 56.32,49.07 and 18.61% (For urea) and 51.61,45.38 and 15.03% (for creatinine) with the rat fed by 4mg.day-1 of 10, 15 and 20% extracts of green coffee, respectively.

Serum lipid profiles

Feeding of the rats orally with green coffee extract (10-20%) by the dose 4mg.day^{-1} prevented the rise of mean serum TG and LDL concentrations. The rate of preventative was increased with the increasing of the extract concentration. Such as shown in Figures (3), the rate of decreasing in TG and LDL concentrations were recorded 57.95,48.49 and 17.87% (For TG) and 56.67,49.10 and 18.64% (for

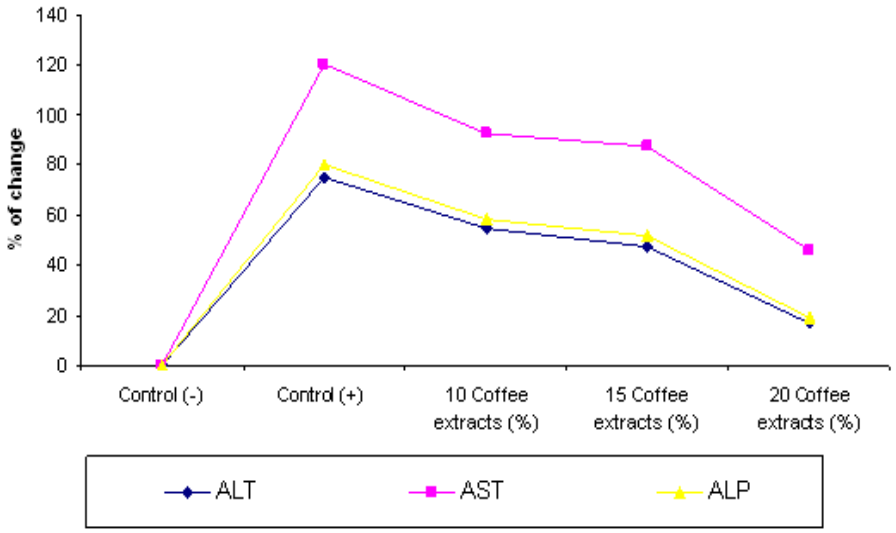

Figure (1): Liver functions (as % of change) of obese-diabetic rats fed orally green coffee beans or rooibos tea extracts

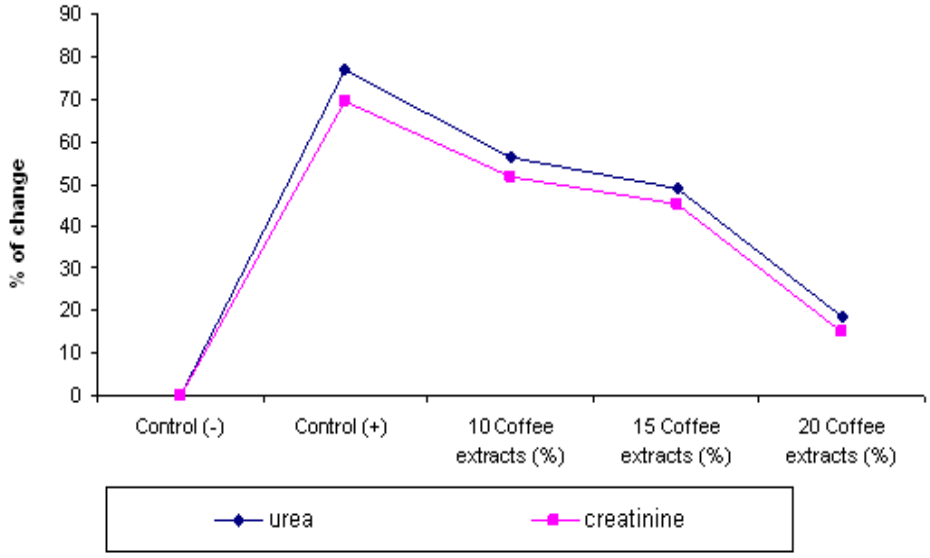

Figure (2): Kidney functions (as % of change) of obese-diabetic rats fed orally coffee or rooibos extracts.

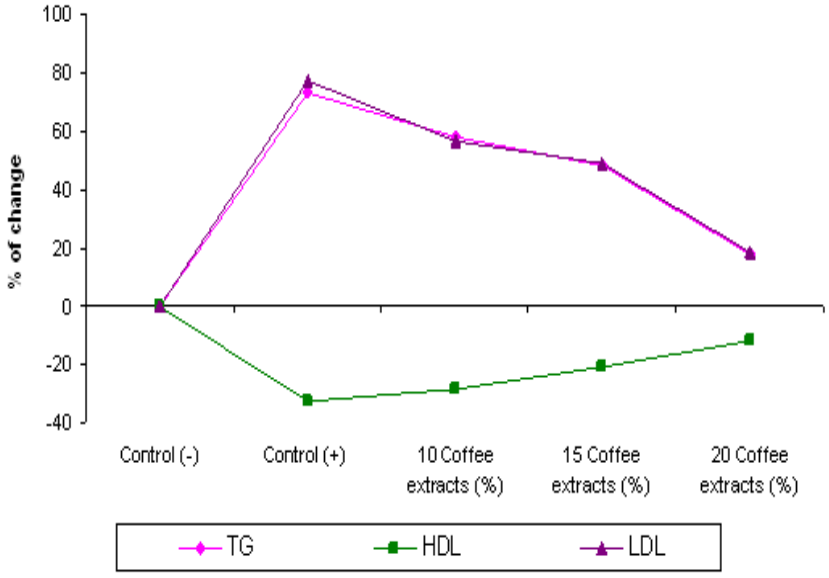

Figure (3): Serum lipid profiles (as % of change) of obese-diabetic rats fed orally coffee or rooibos extracts.

LDL) with the rat fed by 4mg.day^{-1} of 10, 15 and 20% extracts of green coffee, respectively.

Serum lipid peroxidation

Feeding of the rats orally with green coffee extract (10-20%) by the dose 4mg.day^{-1} prevented the rise of mean TBARS concentrations. The rate of preventative was increased with the increasing of the extracts concentration. Such as shown in Figure (4), the rate of decreasing in the TBARS were recorded 81.61,66.52 and 22.52% with the rat injected by 4mg.day-1 of 10, 15 and 20% extracts of green coffee, respectively.

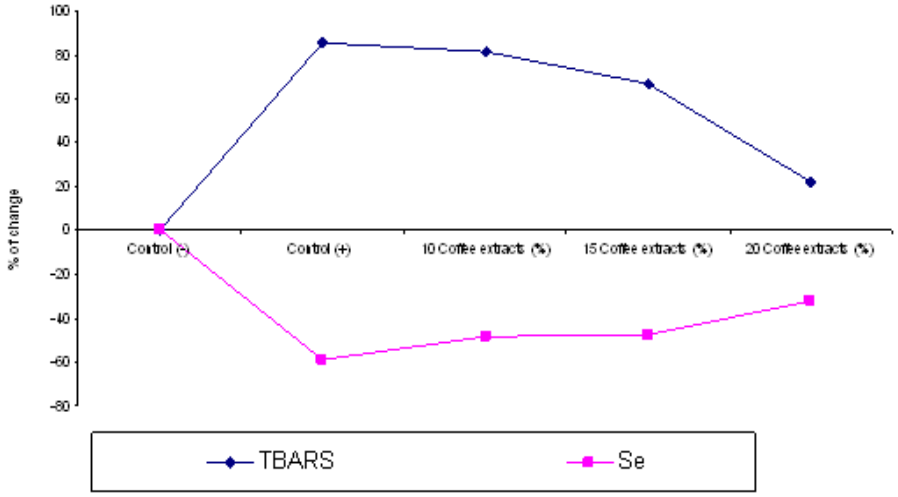

Figure (4): Serum lipid peroxidation (as % of change) of obese-diabetic rats fed orally coffee or rooibos extracts.

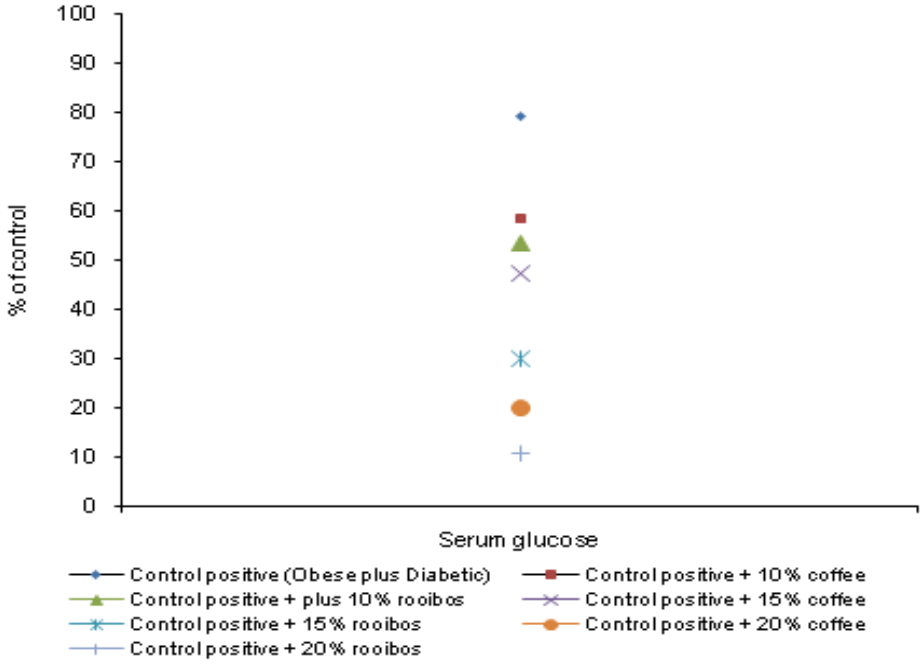

Figure (5): Serum glucose (as % of change) of obese-diabetic rats fed orally coffee or rooibos extracts.

Serum Glucose

Feeding of the rats orally with green coffee extract (10-20%) by the dose 4mg.day^{-1} prevented the rise of mean glucose concentrations. The rate of preventative was increased with the increasing of the extracts concentration. Such as shown in Figures (9-10), the rate of decreasing in the glucose were recorded 58.30,47.28 and 19.92% with the rat injected by 4mg.day-1 of 10, 15 and 20% extracts of green coffee, respectively.

Discussion

There is an inverse relation between Coffee consumption and hepatocellular carcinoma (HCC) regardless of its etiology. Coffee consumption has been inversely related to the serum enzyme activities gamma-glutamyl transferase and alanineamino transferase in studies performed in various countries, In addition, epidemiocological results, taken together indicated that, coffee consumption is inversely related with hepatic cirrhosis, however, they cannot demonstrate a causative role of coffee with prevention of liver injury. Thus coffee drinking may protect against liver injury and lower the risk of liver cancer (Gelatti *et al* ., 2005; and Larsson and Wolk, 2007), Coffee preparations can also efficiently inhibit CCl_4- induced liver fibrosis in rats, the coffee preparations may therefore be a potential functional food for preventing liver fibrosis (Shi *et al* ., 2010). Consumption of regular coffee is an independent protective factor for liver fibrosis in severely obese European patients (Anty *et al*., 2012).

The decreasing in serum uric acid and creatinine as the result of injection plant extract including green coffee could be attributed to their higher content of phytochemicals (Biesalski, 2008).

Coffee lipids may be hypercholesterolaemic and diterpenes could be the lipid component responsible for such an effect (Ratnayake *et al*., 1995). Recent studies have confirmed these findings and elucidated several mechanisms of action including free radical scavenging, metal chelation, inactivation of reactive compounds, and metabolic path way changes (Mori *et al*., 1996 and Cavin *et al*., 2002).

Based on the current findings, highly significant decreasing rate on the formation of TBARS in serum as the result of green coffee extract treatment could be represented an important mode of action of the

antioxidant activity of these plants.These findings agreed with Choi *et al*., (2011), indicated that coffee, contains multiple substances, can promote activities of antioxidant enzymes. The caffeine, chlorogenic acid (CGA), cafestol, trigonelline and kahweol found in coffee are thought to have a significant potential as antioxidants and free radical scavengers (Frost-Meyer and Logomarsino, 2012).

These results agreed with Dorea and da Costa, (2005), reported that coffee might be regarded as a functional food for the prevention of metabolic disease. The possible mode of serum glucose lowering activity of green coffee beans could be explained by one or more of the following process. Bhathena and Valasquez , (2002), reported that Some biologically active ingredients of coffee that were suggested to contribute to its anti diabetic action are Lignans through antioxidant and estrogenic activity.And chlorogenic acid- through reduced glucose absorption and increased production of glucagon-like peptide 1(Mc Carty,2005). Also coffee compounds induced inhibition of 11β-hydroxy steroid dehydrogenase type 1 (11β-HSD1) decreases hepatic gluconeogenesis (Atanasov *et al*., 2006). Therefore regular consumption of caffeinated or decaffeinated coffee beverage exerts a protective effect against type 2 diabetes (Battram *et al* ., 2006).

In conclusion, green coffee extract is effective in protecting against obese and diabetes injuries. These results supported our hypothesis that extracts contains several classes of phytochemicals with other compounds that are able to prevent or inhibit obese and diabetes complications through liver serum enzymes-lowering activity, kidney function parameters concentration lowering, enhancing the serum lipid profile and decreasing rate on the formation of TBARS in serum. Therefore, we recommended like of that plant extracts (green coffee) by a concentration 4 $mg.day^{-1}$ %, amount to be included in our daily diets, drinks and food supplementation.

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دراسات بيولوجية على مستخلص بذور القهوة الخضراء وعلاقته بامراض السمنة وارتفاع السكر فى الدم

يوسف عبد العزيز الحسانين، شريف صبري رجب، وفاء أحمد رفعت ذكي مصطفى
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تم إجراء الدراسة الحالية لدراسة تأثير استهلاك القهوة الخضراء على مرض السمنة والبول السكري. حيث تم استخدام ٢٥ فأر أبيض بالغ يتراوح وزن كلا منها (١٩٠ ±٣٠) جرام وتم تقسيمهم إلى خمس مجموعات متساوية وتركت احداهما كمجموعة ضابطة سالبة , أما المجموعات الاربعة المتبقية تم تغذيتها على غذاء مرتفع في محتواه من الدهون لإصابتها بالسمنة ثم تم إصابتها بالداء السكري عن طريق الحقن بواسطة الالوكسان ١٥٠ملجم/كجم من وزن الجسم داخل الغشاء البريتوني . تم حقن الفئران المصابة بالسمنة والسكري بمستخلص القهوه الخضراء بتركيزات (١٠-٢٠%) بجرعة ٤ مل يوميا عن طريق الفم. وأجريت القياسات التالية إنزيمات الكبد (ALT,AST,ALP)، وظائف الكلي (الكرياتنين واليوريا)، الجليسريدات الثلاثية ودهون الدم (LDL,HDL,T.G)، البيروكسيدات، والجلوكوز في السيرم. وقد أظهرت النتائج تحسنا في كل من وظائف الكبد والكلى ودهون الدم والجلوكوز وانخفاض في بيروكسيدات الدهون لدي فئران التجارب المصابة بالسمنة والبول السكري وتشير هذه النتائج أن مستخلص القهوة الخضراء وشاي الغني في محتواها من الكيماويات النباتية والتي تشمل المواد الفينولية مثل حمض الكلوروجينك. تحسن جلوكوز الدم لدى الفئران المصابة بالسمنة والبول السكري وعلى ذلك فانه يوصى بتناول مستخلصات القهوة الخضراء ضمن الغذاء اليومي.

الكلمات المفتاحية: جلوكوز السيرم، الكيماويات النباتية، وظائف الكبد، وظائف الكلى