Effect of Green Tea (*Camellia sinensis L.*) and L-Carnitine on Male Obese Rat

Radwan, Olfat A.[1], SanaaM. Abdulrahman[2] ,Rania, A. H. Abd El-Aal[3], Abeer A. Zayed[4], and Wafai Z. A. Mikhail[5]

1-Pesticide Analysis Department, Central Agricultural Pesticide Lab (CAPL), Agricultural Research Center

2-Mammalian and Aquatic Toxicology Department, Central Agricultural Pesticide Lab (CAPL), Agricultural Research Center

3-Developmental Pharmacology Department, National Organization for Drug Control & Research.

4-Forensic Medicine & Clinical Toxicology Faculty of Medicine Cairo University.

5-The Department of Natural Resources Institute of African Research& Studies, Cairo University.

Abstract: This work was designed to compare the level of serum glucose and some biochemical parameters in obese rats, obese rats treated with green tea GT or L-Carnitine LC and normal rats. Forty male rats were randomly assigned into four groups, each one consists of 10 rats,the first kept as control (-ve C),the secondserved as positive control (+ve*C*), the third treated with aqueous extract of GT and the fourth treated with L-Carnitine. The aim of the present study is to achieve an 8-week obesity model followed by a 9-week daily treatment with both 10% green tea aqueous extract and 21 mg L-Carnitine / kg body weight. In both third and fourth groups,glucose levels, cholesterol, AST, ALT and ALP were decreased. On contrast, second group (+veC) showed elevation in the previous parameters in comparison with third and fourth groupsand also with normal group (-veC). It is assumed that, the treatment with green tea ameliorate the liver and kidney function. On contrast, the present study revealed that treatment with green tea induces disorder in both liver, kidney functions and structure; these alternations may be attributed to some turbidities and traces of heavy metals which found in green tea.

Keywords: **Green tea, L-Carnitine, Obesity, Biochemical parameter.**

1.Introduction

Obesity is a global phenomenon that has become a public health burden. An increased risk of death, shortening of life and morbidity is associated with obesity (**NAO, 2001**). Obesity is a dynamic multi-factorial chronic disease that arises from a genotype-environment relationship (**WHO, 2004**) and is also considered a major factor in many diseases.

Dayanandan*et al* .(1994) and **Tsuneki*et al* . (2004**) reported that the processing of green tea in diabetic mice produces an antihyperglycemic impact. On the other hand, **Gamal, *et al*. (2009)** postulated that most symptoms of the metabolic syndrome sush, such as hyperglycaemia and hyperlipidaemia, are substantially moderated by tea extracts due to the degradation of pancreatic B cells that secrete insulin and impairment of liver functions.

The activity of L-Carnitine on streptozotocin-induced diabetic rats was investigated by **Uysal, *et al* .(2005)**. Following streptozotocin injection, blood glucose levels were assayed regularly. In male albino rats, L-Carnitine significantly decreased cholesterol , triglycerides, phospholipids, and free fatty acids.

Green tea is very complex with a higher number of polyphenols (epicatechin, epicatechingallate, eipgallocatechin and epigallocatechingallate) compared to black tea, **Belaynesh, *et al* .(2014).**

By inhibiting carbohydrate digesting enzymes and glucose transporters around the intestine, polyphenols reduced carbohydrate digestion and absorption throughout the intestine. It also increases the secretion of insulin by pancreatic beta cells and protects these cells from cytokine-induced inflammatory damage. In addition, green tea assists in the digestion of carbohydrates, increases the activity of insulin and preserves glucose homeostasis.

Tea originates from the Camellia sinensis plant, a tree that, unless cultivated, can grow up to 52 feet in height. Tea plants need 50 inches per year of rainfall and acidic soil. In the soil, air, or water in which the plants are grown, pollutants can differ. Acidic soil can result in aluminium and fluoride being available in excess **(Álvarez-Ayuso, *et al.*, 2011).**

Cadmium (Cd) and lead (Pb) which foun in GT are responsible for significant damage to essential enzymes and multiple body systems, including the circulatory, renal and central nervous systems (**Santos, *et al*., 2013**).

The aim of the current study was to investigate the effect of aqueous green tea extract (GT) and supplemental L-carnitine (LC) on serum glucose, total protein, albumin, globulin, urea, cholesterol, AST, ALT and ALP and the histological structure of albino adult male obese rats in the liver and kidney.

2.Material & Methods

2.1.Plant Material used :

Green tea, *Camellia sinensis* (Ericales: Camellia).green tea package made in China was purchased from local market in Cairo Governorate Green tea extract (GT) was orally administered to rats at a dose of 1 ml/100g body weight daily.

2.2.Drug used:

L-Carnitine (LC) (dietary supplement): 1 ml containing 250 mg carnitine was purchased from the Arab Company for Pharmaceuticals & Medicinal Plants (MEPACO-MEDIFOOD) Enshas El-Raml-Sharkeya –Egypt. Oral administration of a dose of 300 mg/kg body weight daily was done.

2.3.Experimental Animals:

Mature male albino rats (130g±10) were used in the present study. They were kindly supplied by the Egyptian organization of Biological products and vaccines. The animals were bred in appropriate conditions for two weeks to climate before treatments.

2.4.Experimental Design:

A total forty male rats were randomly assigned into two groups, normal 10 male rats and obese thirty male rats. The rats were included in three groups after induction of obesity. In the experiment 10 rats were used in each group. Our goal is to achieve obesity model in 8 weeks by high fat diet feeding, followed by treatment period for 9 weeks. This model provides a reliable method and resembles the clinical cases of obesity and its treatments; also this period of treatment is safe and recommended in previous researches.

2.5.Normal Diet:

Protein 21% + Fats 3.4% + Fibres 3.3%-concentration of Carbohydrate10%+ Yellow maize + soybeans + bone dust as diet complementary.

High Fat Diet (HFD): The HFD contained 20 g of fat/100 g of diet (19 g of butter oil and 1 g of soybean oil to provide essential fatty acids) and provided 19.34 kJ/g of diet, including 7.74 kJ/g as fat**(Stephen *et al.*,2002).**

2.6.Treatment:

Green tea aqueous solution 10% (**El-Sayed&Eslam, 2014),**and L–Carnitine 21mg/kg body weight (**Meky, *et al* ., 2016)**to be used for treated animals are shown in (Table 1) for long term (9 weeks). The administrated solutions were prepared daily.

2.7.Bloodsamples:

By the end of the experimental period(9 weeks), venous blood samples were collected from the orbital sinus of normal, obese control and obese treated groups via glass capillaries at fasting state. Blood samples were collected in a dry tube, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 minutes for separation of serum. The clear, non-haemolysed supernatant sera were separated and stored at -20°C for subsequent biochemical measurements.

Table (1) Green tea (GT) and L-Carnitine (LC) concentrations to be used for treated obese animals.

Group No.	Treatment	Concentration of G.T or LC
Group 1	Control (normal diet)	-
Group 2	Positive Control HFD	-
Group 3	GT administration+ HFD	1ml/100g B.wt.of 10% aqueous solution of G.T
Group 4	LC administration+ HFD	21 mg/kg B.wt of LC.

2.8.Statistical analysis:

Statistical analysis for all data was carried out using analysis of variance (ANOVA) using general linear model program of SAS (SAS 2000). Statistically significant difference among means were set at $p \leq 0.05$ level by using Duncan's Multiple Range .Test (DMRT) procedure **(Duncan, 1955).**

3.Results

3.1.Effects of GT & LC on glucose levels:

Rats treated with (GT & LC) showed decrease in serum glucose level, after 9 weeks of treatment (96.4 & 93.3 mg/100ml) respectively while the serum glucose level in control positive group was increased (118.7 mg/100ml) in comparing with (-ve) control group(**Table2).**

3.2.Effects of GT & LC on cholesterol:

Rats treated with (GT & LC) showed significantdecrease in serum cholesterol level, after 9 weeks of treatment (1.483 & 1.229 mg/100ml) respectively while an elevation in the serum cholesterol level in control positive group was happened (4.410 mg/100ml) comparing with (-ve) control group (3.403 mg/100ml) (**Table, 2).**

Table(2):Effect of GT (aqueous extract) & L- Carnitine on serum glucose , cholesterol and urea levels of male albino rats.

Treatment	Blood glucose mg/100ml	Percentage %	Cholesterol mg/100ml	Percentage %	Urea mg/100ml	Percentage %
Control (-ve)	100±3.13	100%	3.403±0.400	100%	6.942±1.189	100%
Control (+ve)	118.7±2.31*	118.7%	4.410±0.240*	129.59%	5.557±0.927*	80.05%
Green tea	96.4±2.54	96.4%	1.483±0.122*	43.58%	7.875±0.902*	113.44%
L-Carnitine	93.3±1.88	93.3%	1.229±0.0569*	35.85%	7.759±0.323*	104.57%

Values are expressed as means ± standard errors, (Duncan's multiple range test, $p < 0.05$).

3.3.Effects of GT & LC on serum urea:

Rats treated with (GT & LC) showedor exhibited significant increase in serum urea level, after 9 weeks of treatments were (7.875 & 7.759 mg/100ml) respectively while the serum urea level in control positive group was decreased (5.557 mg/100ml) compared with that registeredin (-ve) control group (6.942 mg/100ml) (**Table, 2)**

3.4.Effects of GT & LC on serum total protein:

An administration ofrats with (GT & LC) caused significant increase in serum total protein level, after 9 weeks of treatment (10.390 & 7.682 g/100 ml) respectively while a serum total protein level in control positive group did not (6.859 g/100ml) compared with the first group (6.980 g/100ml) (Table, 3).

3.5.Effects of GT & LC on serum albumin:

Both rats positive control and rats which treated with (GT & LC) showed significant increase in serum albumin level, after 9 weeks of treatments were (1.870 & 1.867&1.744 g/100 ml) respectively compared with the first group which was (1.578 g/100ml) (Table ,3).

3.6.Effects of GT & LC on serum globulin:

Rats treated with (GT & LC) showed significant increase in serum globulin level, after 9 weeks of treatments were (8.523 & 5.938 g/100 ml) respectively while the serum globulin level in control positive group was decreased (4.989 g/100ml) compared with the first group (5.402 g/100ml) (Table 3).

Table (3):Effect of GT (aqueous extract) and L-Carnitine on serum total proteins, albumin and globulin levels of male obese albino rats

Treatment	Total proteins g/100ml	Percentage %	Albumin g/100ml	Percentage %	Globulin g/100ml	Percentage %
Control (-)	6.980±1.457	100%	1.578± 0.240	100%	5.402±1.19	100%
Control (+)	6.859±0.479	98.266%	1.870±0.191*	118.05%	4.989±0.382*	92.35%
Green tea	10.390±0.431 *	148.85%	1.867±0.07*	118.31%	8.523±0.340*	157.77%
L-Carnitine	7.682±0.870*	110.57%	1.744±0.054*	110.52%	5.938*	109.92%

Values are expressed as means ± standard errors, (Duncan's multiple range test, $p < 0.05$).

3.7.Effects of GT & LC on alkaline phosphatase activity:

Treatment ofrats treated with (GT & LC) showed significant decrease in serum alkaline phosphatase. After 9 weeks of treatment(47.826 & 39.193 µl /100ml) respectively while the serum alkaline phosphatase in control positive group was increased (72.796µl /100ml) compared with control in the first group (61.479 µl /100ml) (**Table 4).**

3.8.Effects of GT & LC on aspartate aminotransferase (AST) activity:

Rats treated with (GT& LC) had significant decrease in serum AST, after 9 weeks of treatments were (44.773& 53.878 µl /100ml) respectively while the AST in control positive group was increased (109.254µl /100ml) compared with control in the first group which was (60.587µl /100ml) (**Table 4).**

3.9.Effects of GT & LC on aspartate aminotransferase (ALT) activity:

Rats treated with (GT & LC) had significant decreases in serum ALT. After 9 weeks of treatments were (44.075 & 44.415 µl /100ml) respectively while the ALT in control positive group was increased (93.52 µl /100ml) compared with control in the first group which was (61.55 µl /100ml)(**Table, 4).**

3.10.Effects of GT & LC on body weight % in obese rats:

Depicts the progression of body weight percentage in the four groups of animals. Effect of aqueous extract prepared from green tea and L-Carnitine on body weight gain % of obese rats presented in Table (2). The mean of body weight gain % of the positive control group increased as compared to the negative control group (74.1% and 72.3%), respectively. the progression of body weight % of two treated groups with green tea aqueous extracts and treated group treated with L-Carnitine showed decreased in the progression of body weight % ompared with positive and negative control groups (74.1 % and 72.3%) respectively. While the progression of body weight % in group which treated with L-Carnitine (53.2%)was more decreased which is treated with green tea extract (61.3%) **(Table 5)**.

3.11. HISTOLOGICAL RESULTS:

Kidney of rat from control normal group showing normal histological structure of renal parenchyma (Fig.1). Kidney of rat from group L-C showing atrophy of glomerular tuft and distension of Bowman's space (Fig.2). sections of rat was treated with GT revealed cytoplasmic vacuolation of epithelial lining renal tubules (Fig.3) and cystic dilatation of renal tubules with protein casts (Fig.4). Figure (5) showed

Table (4): Effect of GT (aqueous extract) and L-Carnitine on serum ALT, AST and alkaline phosphatase in male albino rats.

Treatment	AST µ/ml	Percentage %	ALTµ/ml	Percentage %	Alkaline phosphatase µl /100ml	Percentage %
Control (-)	60.587±7.632	100%	61.55±6.648	100%	61.479±4.828	100%
Control (+)	109.254±9.542	180.33%*	93.52±1.944	151.94%	72.796±5.660*	117.80%
Green tea	44.773±7.689	73.899%*	44.075±2.487	71.61%	47.826±1.856*	77.79%
L-Carnitine	53.878±3.277	88.93%*	44.415±8.788	72.16%	39.193±3.035*	63.75%

Values are expressed as means ± standard errors, (Duncan's multiple range test, $p < 0.05$).

Table (5): Percentage of body weight (BW) gain of male albino rats administrated with Green Tea extract (GT) and L-Carnitine (LC) with high fat diet (HFD) for 9 weeks.

Week / Group	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
G1 Control (-)	11.6	17.9	27.5	38.1	46.8	57.1	63.4	69.6	72.3
G2 Control (+)	14.3	20.8	30.9	42.7	53.4	59.8	65.8	72.8	74.1
G3 GT Treatment	14.2	20.3	29.7	40.1	49.6	54.3	56.9	59.8	61.3
G4LC Treatment	10.05	12.9	21.8	30.4	36.4	39.9	45.9	50.1	**53.2**

normal histological structure of liver of control group. Liver of rat treated orally with LC showed congestion of central vein and necrosis of sporadic hepatocytes (Fig.6). Examination of liver of rat treated with GT revealed slight vacuolar degeneration of hepatocytes (Fig.7) and fibroplasia in the portal triad associated with appearance of newly formed bile ductuoles, in addition to congestion of central vein and necrosis of sporadic hepatocytes (Fig.8).

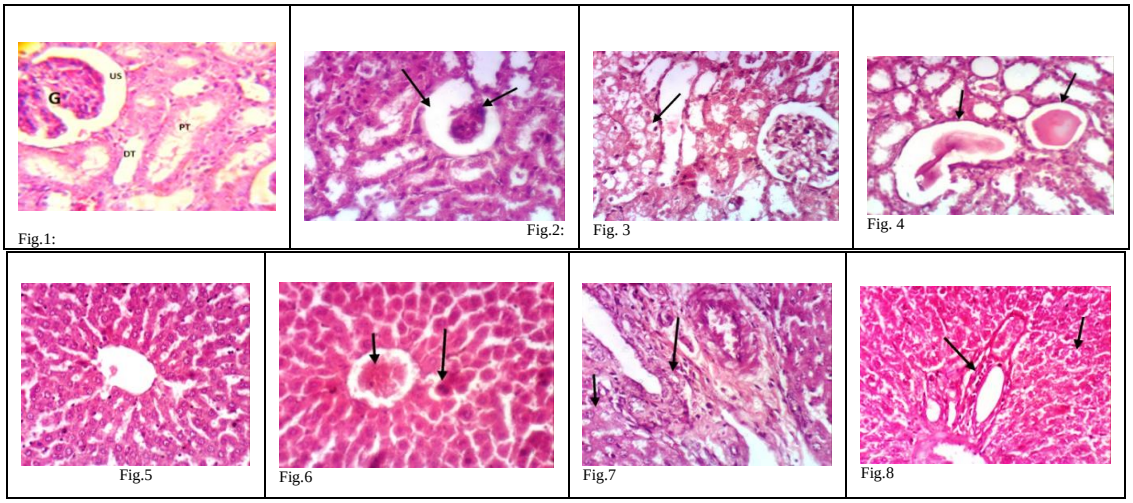

Fig.1: Fig.2: Fig. 3 Fig. 4

Fig.5 Fig.6 Fig.7 Fig.8

Fig.1: Kidney of rat from control group showing normal histological structure of renal parenchyma (H & E X 400).Fig. (2): Kidney of rat from group L-C showing atrophy of glomerular tuft and distension of Bowman's space (H & E X 400).Fig. (3): Kidney of male albino rat from green tea group showing cytoplasmic vacuolation of epithelial lining renal tubules (H & E X 400).Fig. (4): Kidney of rat from group GT showing cystic dilatation of renal tubules with protein casts (H & E X 400).Fig. (5): Liver of rat from control group showing the normal histological structure of hepatic lobule (H & E X 400).Fig. (6): Liver of rat from L-C group showing congestion of central vein and necrosis of sporadic hepatocytes (H & E X 400).Fig. (7): Liver of rat from GT group showing slight vacuolar degeneration of hepatocytes and fibroplasia in the portal triad associated with appearance of newly formed bile ductuoles (H & E X 400) Fig. (8): Liver of rat from GT group showing slight hydropic degeneration of hepatocytes and slight fibroplasia in the portal triad (H & E X 400)

4.Discussion

In the current research, the use of green tea aqueous extract (GT) (10 %) and L-carnitine (LC) in obese rats (10 % GT) at the prescribed dose (21 mg LC / kg body weight) decreased serum glucose levels in adult obese male albino rats (GT and LC) compared to control (C+ve) in both treatments.

These results are consistent with previous research **(Murase, *et al.*, 2002; Ene, *et al.*, 2008; Kasetti, *et al.* , 2010; Sudathip*et al.*, 2012; Haidari *et al.*, 2012).** Obesity, metabolic syndrome, hepatic steatosis and the release of hepatic enzymes caused by a high-fat diet in mice were inhibited by green tea epigallocatechingallate**(Bose, *et al.*, 2008).**

Muoio, *et al.*, 2012; Gary, 2016; Mansour *et al.*(2017) showed that L-Carnitine has remarkable anti-diabetic effects and is likely to be associated with antioxidant activity of L-Carnitine.

The high levels of polyphenols in green tea are important for the prevention of various diseases. Green tea has so far been shown to have many effects on the metabolism of carbohydrates and is considered an alternative treatment for diabetes mellitus **(Belaynesh, *et al*., 2014).**

The effect of green tea extract (GTE) on liver and kidney functions in aged rats was investigated by **Gad &Zaghloul (2013).** Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activity decreased significantly in the treatment of aged rats with GTE.

The effect of Green Tea Leaf Extract (GTLE) on the functional and morphological male reproductive system was studied by **Das and Karmakar (2015).** Two different study animal groups with two different doses were given the extract for 26 consecutive days. Following surgery, the weight of the testis was substantially reduced relative to the usual weight gain of all the animals. Compared with control, the sperm count was decreased in the treated classes. In the treated classes, the testosterone level was decreased. The concentrations of FSH and LH in the treated groups were different. The results of this study showed that GTLE has a dose-dependent, potent castrative effect on the male reproductive system.

In the liver of adult male Wistar albino rats, **Zaki *et al*. (2017)** investigated the subchronic toxicity of GTE. The rats were divided into four groups: group I (control), group II (low green tea dose), group III (medium green tea dose) and group IV (high green tea dose), respectively. Histopathological and histomorphometric studies have been performed. There were congested core veins and hepatic sinusoids. Hepatocyte degeneration, hepatic artery hypertrophy, dilation of the bile ducts, and cellular infiltration were clearly observed.

Dental or skeletal fluorosis can be the result of consuming more than 5 litres of tea per week **(Lung, *et al*, 2008).**

With 73 percent of teas brewed for 3 minutes and 83 percent brewed for 15 minutes, all brewed teas containing lead have lead levels deemed unsafe for pregnancy and lactation use **(Shekoohiyan, *et al*., 2012).**

Lead, arsenic, aluminium, and cadmium are among the poisonous elements. Accumulated in the brain and consequent impairment of cognitive growth are the extremely low levels of lead recognised during the prenatal period, **Genuis, (2011).** The intake of this and some prenatal vitamins can easily exceed this daily limit and lead, especially in the foetus, to substantial bioaccumulation over time **(Genuis, *et al*., 2012).**

On the other hand, due to a reduction in body and liver fats by enhancing lipid metabolism, increasing energy consumption, and influencing fat absorption and excretion, the substantial decrease in body weight gain. The findings therefore indicate a clear influence of tea extracts on enhancing metabolism rather than influencing the intake of food **(Gamal, *et al*., 2009).**

These results are consistent with previous work showing that green tea and carnitine have a potential role in regulating body weight. But the decrease in body weight gain was lower for male albino rats that administered GT than for albino rats that administered LC. In L-carnitine treated animals, the rise in weight loss could be greater than the weight loss in fatty animals treated with green tea.

The biochemical findings are verified by histological tests on tissues. In the liver, GT indicated vacuolar hepatocyte degeneration, portal triad fibroplasia, and necrosis. These findings were consistent with a decline in ALT&AST liver enzymes. Cytoplasmic vacuolation of epithelial lining renal tubules and cystic dilatation of protein cast renal tubules were observed in the kidneys of rats treated with GT. After treatment with GT, all kidney functions improved. All of these lesions that occur in both liver and kidney may attribute to heavy metals which found in GT.

Conclusion

It was concluded that GT contains high amount of polyphenols therefore,it is known to have a preventive effect against cancer. On the other hand, GT also contain some heavy metals which represent severity on health. It was recommended very careful using of GT.

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تأثير الشاي الأخضر ول - كارنيتين على ذكر الجرذان البدين

ألفت رضوان[١] سناء عبدالرحمن[٢] ، رانيا ، عبد الحميد عبد العال[٣] ، عبير زايد[٤] ، وفائى ميخائيل[٥].

١- المعمل المركزي للمبيدات الزراعية بمركز البحوث الزراعية

٢- قسم السموم الثديية والمائية ، المعمل المركزى للمبيدات الزراعة المركزي

٣- قسم الصيدلة التنموية - الهيئة القومية للرقابة والبحوث الدوائية.

٤- قسم الطب الشرعي وعلم السموم الاكلينيكي بكلية الطب جامعة القاهرة.

٥- بقسم الموارد الطبيعية ،معهد البحوث والدراسات الأفريقية ، جامعة القاهرة.

الملخص العربي:

صمم هذا العمل لمقارنة مستوى الجلوكوز في الدم وبعض المتغيرات البيوكيميائية في الجرذان البدينة ، الفئران البدينة المعالجة بالشاي الأخضر أو ل - كارنيتينوالفئران العادية. تم تقسيم أربعين من ذكور الجرذان بشكل عشوائي إلى أربع مجموعات ، كل مجموعة تتكون من ١٠ جرذان، المجموعة الأولى تم الاحتفاظ بها كمجموعة ضابطة(VC-) ، والثانية كانت بمثابة تحكم إيجابي (veC+) ، والثالثة تمت معالجتها بمستخلص مائي من GT والرابعة يعالج بـ L-Carnitine. الهدف من هذه الدراسة هو تحقيق نموذج السمنة لمدة ٨ أسابيع متبوعًا بعلاج يومي لمدة ٩ أسابيع مع كل من مستخلص الشاي الأخضر المائي بنسبة ١٠٪ و ٢١ مجم/ كجم منL-Carnitine من وزن الجسم. في كلتا المجموعتين الثالثة والرابعة ، انخفضت مستويات الجلوكوز والكوليسترول و AST و ALT و ALP.

على النقيض من ذلك ، أظهرت المجموعة الثانية (veC+) ارتفاعًا في المعدلات السابقة مقارنة بالمجموعتين الثالثة والرابعة وأيضًا مع المجموعة الطبيعية (veC-). من المفترض أن العلاج بالشاي الأخضر يحسن وظائف الكبد والكلى. على النقيض من ذلك ، كشفت الدراسة الحالية أن العلاج بالشاي الأخضر يؤدي إلى اضطراب في وظائف الكبد والكلى وهيكلهما. يمكن أن تعزى هذه التغيرات إلى المعادن الثقيلة الموجودة في الشاي الأخضر.