

## Effect of sweet chestnut (*Castanea sativa*) in streptozotocin induced diabetic rats

Dalia R. Hassan\* and Salem A. Salem

Department of Home Economic, Faculty of Specific Education, Fayoum – University

\*[dr.dal.refaat@gmail.com](mailto:dr.dal.refaat@gmail.com)

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### ABSTRACT

Diabetic chronic hyperglycemia is connected with organ deterioration, malfunction, and failure, including the kidneys. The current study explores the positive effects of chestnut (*Castanea sativa*) therapy on oxidative stress and renal function in streptozotocin "STZ" induced diabetic rats, as well as assesses the sensory properties of baked cookies containing varying doses of chestnuts. Thirty-five rats were used in this study and were divided into the following 5; group (1) or (-ve control) provided a baseline diet, and the remaining were injected with streptozotocin "STZ" and fed experimental diets for four weeks after the diabetes mellitus "DM" was confirmed. Group (2) or (+ve control) includes DM-rats without treatment; groups (3,4 & 5) DM-rats treated with 5, 10 and 15% CS, respectively. At the ending period, samples of blood were collected for measuring levels of blood glucose, serum HB1C, uric acid, creatinine and urea levels, malondialdehyde, superoxide dismutase "SOD", and glutathione levels "GSH", Results indicated that STZ induced diabetic rats (DM-positive) showed highly significant ( $P < 0.01$ ) increase in their blood glucose, MDA, serum urea, serum creatinine, and serum uric acid and a highly significant decrease ( $P < 0.01$ ) in GSH and SOD levels. DM rats fed CS had a significant decrease in their serum blood glucose, MDA, creatinine, and uric acid and an increase in GSH and SOD levels. Therefore, CS can be used in controlling diabetes and its harmful consequences and to enhance the antioxidant status and renal protective effects in diabetic rats. Also, Sensory evaluation results revealed that cookies prepared with CS at 5%, 10%, and 15 % were accepted by panellists with different ratings.

**Keywords:** Sweet chestnut, *Castanea sativa*, diabetic rats, Phenolic compounds.

### INTRODUCTION

Kidneys maintain homeostasis in the animal body through the regulation the composition of fluids and excretion of produced metabolic wastes (Ming and Ian, 2019), hence diabetes being a major cause of chronic kidney disease. The elevated reactive oxygen species "ROS" and inadequate antioxidant activity are related to Diabetes mellitus "DM" (Shanmugam *et al.*, 2011). Furthermore, both diabetes T1 and T2 are linked to oxidative stress (Rackova *et al.*, (2013), and studies have linked oxidative stress to various forms of renal damage and nephrotoxicity. Researchers have been looking for insulin-like compounds derived from plants to

treat diabetes (Rafiq *et al.*, 2009). Diabetes-related persistent hyperglycemia is linked to organ damage and failure, including the kidneys, therefore it is essential to search for functional food by which oxidative stress may be ameliorated in diabetic. Additionally, due to health issues like diabetes that are linked to food intake, as well as the fact that cookies may be used to deliver crucial nutrients to the population, there is an increased push for the consumption of functional foods (Chinma and Gernah, 2007).

The chestnut "*Castanea sativa*" is a fruit belonging to Family Fagaceae and is grown in most Mediterranean Europe countries (Míguez *et al.*, 2019). It is one of

bioactive tannins phenolic compounds (Sanz *et al.*, 2010). Therefore, there was a great consumption of chestnut due to their nutritional values. The chestnuts offer unusual nutritional features, such as considerable amounts of dietary fibres and carbohydrate, but low levels of protein "2-4%" and fat "2-5% mainly unsaturated fatty acids (Akbulut *et al.*, 2017; Otlis and Selek, 2012) and it is a source of energy with several health advantages. The polyphenolic and organic components of the fruits also have a strong antioxidant action (Gonçalves *et al.*, 2010).

Due to CS nutritional value and health advantages, it has a significant role in the human diet. For instance, they can help reduce abdominal obesity and can be used in gluten-free diets for those with celiac disease (El Khoury *et al.*, 2018). Chestnuts now have significant potential as functional foods or food additives due to the rising desire for traditional meals. The nuts are used to make various goods with added value for the cake and confectionery industries (Mert and Ertürk, 2017). Given that roasted chestnuts have minimal fat content and are an excellent source of phenolics "gallic and ellagic acids" and citric acid (Gonçalves *et al.*, 2010).

Therefore the purpose of this study is to investigate and evaluate the importance of using the sweet chestnut "*C. sativa*" in foods to reduce or treat hyperglycemia, lipid peroxidation, antioxidant status and renal function in hyperglycemic rats. Also, to evaluate the color, taste, aroma, texture and overall quality of using the chestnuts *C. sativa* to prepare cookies.

## MATERIALS AND METHODS

### Materials:

Chestnut "*C. sativa*" was obtained from the local market

Streptozotocin was obtained from Sigma Aldrich (St. Louis, MO).

Rats: Thirty five male albino rats of Sprague Dawely weighing 150- 180g were

obtained from the Egyptian Organization for Biological Products and Vaccines "VACSERA"

### Methods:

#### Preparation of Chestnut powder

*C. sativa* was washed by distilled water, and then it was dried at 40°C in an oven, ground, and refrigerated in an airtight container.

**Preparation of treated diet:** it was prepared by adding of dry chestnuts powder at 5%, 10% and 15%/kg baseline diet (corn starch) (According to Reeves *et al.*, 1993).

#### Preparation of experimental groups:

The experimental 35 male albino rats were contained individually in cages at 25°C. Rats were divided into 5 groups "Seven rats in each group" and meal was given to the rats in a unique feeding cup to minimise food spillage.

Diabetes Mellitus (DM) was induced by means of a single intraperitoneally (I.P.) injection of rats with 40mg Streptozotocin/kg body weight for 5 consecutive days according to the method of Jelena *et al.* (2017). After receiving I.P. injections, the animals were given 5% glucose solution to prevent hypoglycemia shock-related mortality. To confirm the induction of diabetes, blood samples were taken from rats 72 hours later. The blood glucose concentration was then determined, the non-diabetic rats were removed from the study, and diabetes was confirmed by measuring level of non-fasting blood glucose (>250 mg/dl).

Rats of normal control group (1): was kept on basal diet feeding and the remaining DM-rats were separated into 4 groups, each of rats received the experimental diets for 4 weeks after diabetes mellitus was confirmed.

The DM- rats' groups were divided as follows;

Group (2): DM-rats positive control fed basal diet;

## Effect of sweet chestnut (*Castanea sativa*) in streptozotocin induced diabetic rats

Group (3): DM-rats feed *C. sativa* 5%/kg diet;

Group (4): DM- feed *C. sativa* 10%/kg diet;

Group(5): DM- feed *C. sativa* 15%/kg diet.

### Biochemical analysis

After period of 4 weeks, rats were stupefied using diethyl ether and samples of blood were obtained from the hepatic portal vein. One part of the blood was put in a heparinized tube to measure blood glucose, the remaining blood samples were collected and Serum was separated by centrifuging at 3000 rpm for 15 minutes, and it was then kept at  $-20^{\circ}$  C for biochemical analysis. The total phenolic and phenolic compounds were determined by Spectrophotometric.

Using gallic acid as a reference material, the total content of polyphenol "TP" was assessed using the Folin-Ciocalteu colorimetric technique (Ciucure *et al.*, 2019). Gallic acid was determined by spectrometry. Blood glucose concentration was assessed according to Yenson (1986). The level of serum glutathione "GSH" was determined according to Beutler *et al.* (1963), while malondialdehyde "MDA" was assessed according to Esterbauer *et al.* (1991). The method of Caraway (1955) was applied to measure the serum uric acid, while the level of creatinine was determined after (Bohmer, 1971) and urea level was measured after (Marsch *et al.*, 1965).

### Sensory Evaluation of the (CS) cookies

The control cookies was prepared after kiin and Eke (2013), while the amounts of components used in cookie making used in treatment of DM rats were somewhat modified from those stated by Chinma *et al.* (2011) and Kiin and Eki (2013). The materials used in preparation

of these cookies include flour "100gm", sugar "53.0g", margarine "26.5g", sodium bicarbonate "1.10g", sodium chloride "0.89g", unsweetened evaporated liquid milk "7.5ml " and water "12.0ml", with a varied quantity of CS as wheat flower expense. The tested cookies containing 50, 100 and 150 g of dry CS g /kg diet were subjected to organoleptic evaluation.

Panelists (10 members) were asked to rank various samples for color, taste, aroma, texture and overall quality on a scale of A 9-point hedonic scale (Moretti *et al.*, 2004).

### Statistical analysis:

The significance differences between the different groups were estimated using One-way analysis of variance. The significance of differences between the means were calculated using the Least Significant Difference "LSD" test. The SPSS programme was used for all data analysis

## RESULT AND DISCUSSION

It was obvious from Table (1) that *Castanea sativa* contains  $321 \pm 30$ ug/mg total phenol compound and also has high levels of tannin and Gallic acid ( $35 \pm 50$  and  $1.670 \pm 0.57$ mg/g, respectively) which could explain its remarkable importance. This result was in agreement with that of Baraga (2015) who indicated that, the primary phenolic chemicals found in chestnut include flavonoids (quercetin derivatives), tannins, and phenolic acids, particularly Gallic acids. Also, Vanessa *et al.* (2020) indicated that the chestnut byproducts have economical benefit where it was used as coadjutors to antibiotics, nutraceutical additives, and antioxidant additives.

**Table (1). Phenolic compounds of chestnut (*Castanea sativa*).**

Sample	Total Phenol Content	Total Content of Tannin	Gallic acid
Inner Shell	$321 \pm 30$ ug/mg	$35 \pm 50$ mg/g	$1.670 \pm 0.57$ mg/g

A variety of biological actions, such as antioxidant, chemo preventive, anti-inflammatory, neuroprotective, and cardio protective properties are exhibited by many natural substances. It is recognized that the sweet chestnut (*C. sativa*) contains phenolic bioactive chemicals, particularly tannins (Sanz *et al.*, 2010). In addition, Mujic (2011) found that CS had a strong antioxidant impact that was correlated with its high phenolic

and flavonoid component content which greatly improved redox state and significantly boosted in vitro-cell survival.

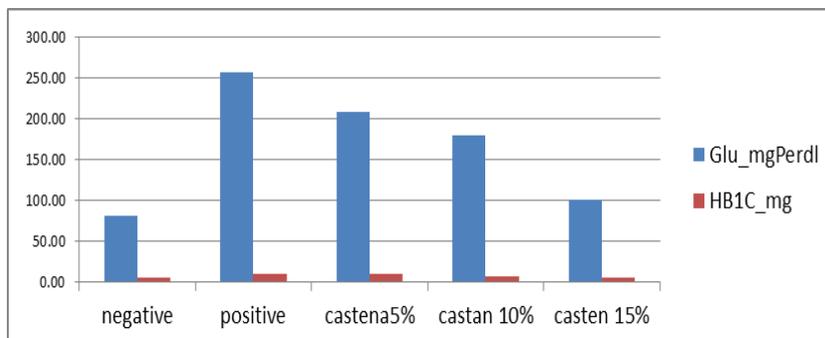
It was noticed from Table (2) and Figures (1 &2) that STZ induced diabetic rats (DM-positive control) have great significant ( $P < 0.01$ ) increase in their blood glucose, HB1C, serum malondialdehyde, urea, creatinine and uric acid (256.8, 10.6, 13.43, 46, 4.39, and 10.79mg/dl, respectively) when compared with the normal negative group (group 1).

**Table (2). Serum glucose, HB1C, urea, creatinine, uric acid (mg/dl) in all rats groups.**

Group	Glu (mg/dl)	HB1C (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
Negative Control	81.97 ± 1.53 **	4.63 ± 0.23**	22.63 ± 0.71**	0.57 ± 0.06**	2.94 ± 0.19**
Positive Control	256.80 ± 5.56	10.60 ± 0.33	46.00 ± 0.63	4.39 ± 0.17	10.79 ± 0.27
<i>C. sativa</i> 5%	208.40 ± 2.42 **	9.20 ± 0.24**	41.50 ± 0.58**	3.38 ± 0.17**	7.86 ± 0.25**
<i>C. sativa</i> 10%	180.28 ± 2.26**	7.48 ± 0.20**	36.92 ± 1.12**	2.79 ± 0.13**	6.42 ± 0.24**
<i>C. sativa</i> 15%	100.98 ± 1.43**	5.45 ± 0.36**	22.60 ± 0.74**	1.75 ± 0.09**	3.48 ± 0.26**

\*Significant differences from Positive Group at ( $P \leq 0.05$ )

\*\*Significant differences from Positive Group at ( $P \leq 0.01$ )



**Fig. (1). Serum glucose and HB1C in all rats groups.**

**Fig. (2). Serum levels of urea, creatinine and uric acid in all rats groups.**

On the other hand, Table (3) revealed that there was a great significant decrease ( $P < 0.01$ ) in levels of serum

GSH, SOD by 40.5% and 17.4%, respectively in DM-positive control group when compared with normal group. This

### Effect of sweet chestnut (*Castanea sativa*) in streptozotocin induced diabetic rats

result may be explained on the basis that, streptozotocin (STZ) is especially harmful to the pancreas, which causes the Langerhans islets' B-cells to degenerate (Hayash *et al.*, 2006).

On the other hand, rats fed on CS had a creatinine value that is an indicator of renal safety and ranged from 3.38 mg/dl in STZ + CS 5% (Group 3) to 1.75 mg/dl in STZ + CS 15% (Group 5), while creatinine value in rats of positive control was 4.39 mg/dl (Table 2). This indicates the effect of CS phenolic compound in lower serum creatinine.

Rats of DM-positive group have significant high at ( $P < 0.01$ ) in MDA levels comparing to control group. This was in harmony with the result of Quine and Raghu (2005) who indicated that in diabetic rats the lipid peroxide product "MDA" is determined as an index of the increased lipid peroxidation where the increase in oxidative stress in certain tissues can lead to the increase in the lipid peroxidation rate., Morakinyo *et al.* (2011) showed that, STZ- diabetic rats had an increased level of MDA, and this could be due to the increase in ROS which is implicated in the progression of hyperglycemia (Suryanarayana *et al.*, 2007).

In the present study the level of blood glucose was significantly increased ( $P \leq 0.01$ ) in the STZ group (256.8 mg/dl  $\pm$  5.5 mg/dl). Treatment of diabetic rats with CS significantly reduced ( $P \leq 0.01$ ) the glucose level and this improves the antioxidant properties which could inhibit cytotoxic signaling pathways and minimizing the liver-damaging consequences of diabetic rats. Ramasamy (2006) and; Mihailovic *et al.* (2013) found that CS has an antiglycation effects and offer a strong therapeutic possibility for the treatment of diabetes.

In the present results indicated that the activity of antioxidant enzymes, SOD and GSH, was decreased in diabetic rats and using CS recovered the antioxidant enzymes activities. In diabetic rats,

antioxidant enzyme activity was reduced, and the associated gene expression was also lower. Mihailović (2020) reported that CS phenolic compound could affect antioxidant especially SOD and affect on lowering blood glucose by its content of phenolic compound. The high phenolic and flavonoid contents of CS reported in the current study may be responsible for its anti-hyperglycemic activity.

Also, the values of urea in rats fed on CS ranged from 41.50 mg/dl in STZ + CS 5% group to 22.60 mg/dl in STZ + CS 15% group, while rat with STZ as a positive control was 46.00 mg/dl was highly significant when compared with negative group.

Data in Table (3) and Figure (3) revealed that the values of GSH and SOD in MD rat fed on CS varying from 8.14 to 14.01 mg/dl and from 2.99 to 4.69 mg/dl in groups (3 & 5, respectively). This explains how CS influences oxidative status.

Lyra *et al.* (2006) reported that chronic hyperglycemia causes oxidative damage in tissues and organs in STZ-induced diabetic rats via producing ROS. The considerable declines in activity of the antioxidant enzyme and the GSH/GSSG ratio indicate that the liver and renal dysfunction was caused by failure of the antioxidant defense systems to keep redox equilibrium (Mihailovic *et al.*, 2015).

The oxidative stress plays a role in death of  $\beta$ -cell or malfunction, which eventually leads to produce diabetes. Lipid peroxidation reveals irreversible oxidative alterations in membranes that affect cell function (Milei *et al.*, 2007). In the current study when compared to the positive control group (group 2), the STZ treatment increased the lipid peroxidation level.

The present results indicated that levels of MDA in rats injected with STZ was higher than that in STZ rats and treated with different CS levels. This demonstrated that CS reduced lipid peroxidation significantly and chestnut exhibited the greatest possibility for lipid peroxidation prevention.

Apart from a general improvement in oxidative state, the individual effects of CS on survival of cells were observed. The CS had a great influence on SOD activity. The decrease in STZ-induced elevation of Larrosa *et al.* (2010) reported that Mn SOD activities following *C. sativa* administration is most likely due to a reduction in levels of ROS due to the strong scavenging ability of CS against ROS.

From Figures (1, 2 & 3) it could be noticed that, STZ induced diabetic rats (DM-positive group) have highly significant increase in blood glucose, creatinine, MDA, urea and uric acid when compared with normal group (group 1). On the other hand, there were highly significant ( $p < 0.05$ ) reduction in SOD and GSH in DM-positive control group when compared with normal control group.

These results may be explained on the basis that (STZ) is toxic to the pancreas, and STZ injection causes Langerhans islet B-cell deterioration (Hayashi *et al.*, 2006).

Results in Figure (3) showed that, rats of DM-positive group have significantly higher MDA levels ( $P < 0.05$ ) comparing to that of control group. This was consistent with the findings of Quine and Raghu (2005), who demonstrated that MDA production is evaluated and this is an indication of enhanced lipid peroxidation in diabetic rats. Furthermore, Morakinyo *et al.* (2011) discovered that STZ- diabetic rats had higher levels of MDA, which might be attributed to an increase in ROS, which is implicated in the development of hyperglycemia (Suryanarayana *et al.*, 2007).

**Table (3): Serum antioxidant enzymes (MDA, GSH and SOD) in all rats groups.**

	MDA nmol/ml	GSH mg/dl	SOD u/ml
<b>Negative Control</b>	2.20 ± 0.11**	16.43 ± 0.36**	5.06 ± 0.13**
<b>Positive Control</b>	13.43 ± 0.30	6.67 ± 0.23	0.88 ± 0.08
<b><i>C. sativa</i> 5%</b>	12.91 ± 0.35	8.14 ± 0.15**	2.99 ± 0.15**
<b><i>C. sativa</i> 10%</b>	8.12 ± 0.29**	8.97 ± 0.20**	3.49 ± 0.15**
<b><i>C. sativa</i> 15%</b>	4.42 ± 0.21**	14.01 ± 0.36**	4.69 ± 0.13**

\*Significant differences from Positive G at  $P \leq 0.05$

\*\*Significant differences from Positive G at  $P \leq 0.01$

**Fig. (3). Serum antioxidant enzyme (MDA,SOD,GSH)in all rats groups**

#### Sensory evaluation:

Data in Table (4) revealed that colour, taste, appearance of cookies with

15% CS were similar to control cookies with wheat flour and ranking the highest of all other CS levels.

**Table (4).** Colour, taste, texture, appearance and overall quality of CS cookies at different levels.

	Color	Appearance	Texture	Taste	Overall Quality
control	9.8	9.7	9.6	8.9	9.7
cook 5% <i>C. sativa</i>	9.1	8.3	7.7	7.8	9.1
cook 10% <i>C. sativa</i>	8.41	8.83	7.47	6.98	9
cook 15% <i>C. sativa</i>	9.9	9.6	8.6	8.7	9.3

It is widely known that high moisture content in a flour sample suggests higher susceptibility to spoiling and, as a result, a shorter shelf life (Othi *et al.*, 2014). As a result, including chestnuts into cookies may prolong shelf life and improve nut palatability, which is not acceptable for subjects to take advantage of.

Soronja *et al.* (2017) indicated that chestnut flour includes sucrose that influences the rheological characteristics of bread goods by preventing the hydration of starch granules and starch gelatinization.

In the present study, using CS as a replacement for wheat flour increased the redness of the cookie samples. According to Dall' *et al.* (2013) CS had a greater influence on biscuits colour.

Baking caused caramelization and Maillard reactions which are linked to CS flour due to its comparatively high sugar (20-32%) and starch (50-60%) content (Paciulli *et al.*, 2018).

The colour and appearance of biscuits made with CS flour were rated higher than those of cookies made with wheat flour (Table 4). The positive impact of CS flour to qualities like as appearance, shape, crumb structure, and so on was consistent with earlier research (Soronja *et al.*, 2017).

### Conclusion

The current study shade light on the phytochemicals which provided by sweet chestnuts and their importance to be included in the diabetic patients diets due to its highly content of phenolic compounds which implicated on prevention the lipid peroxidation and

prevent lowering antioxidant activity and taste good in cookies.

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### Effect of sweet chestnut (*Castanea sativa*) in streptozotocin induced diabetic rats

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تأثير الكستناء الحلو (*Castanea sativa*) في الجرذان المصابة بداء السكري التي يسببها الستربتوزوتوسين

داليارفعت حسن\* ، سالم على سالم  
قسم الاقتصاد المنزلي، كلية التربية النوعية، جامعة الفيوم  
[\\*dr.dal.refaat@gmail.com](mailto:dr.dal.refaat@gmail.com)

### المستخلص

يرتبط ارتفاع السكر المزمن في الدم بتدهور الأعضاء ، والخلل الوظيفي ، بما في ذلك فشل الكلى. سعت الدراسة الحالية إلى اكتشاف الآثار الإيجابية للعلاج بالكستناء الحلو (*Castanea sativa*) على الإجهاد التأكسدي وتأثيره على وظيفة الكلى في نموذج الجرذان المصابة بداء السكري المستحدث عن طريق حقن مادة الستربتوزوتوسين (STZ) ، وكذلك لتقييم الخصائص الحسية للمخبوزات التي تحتوي على مستويات مختلفة من الكستناء الحلو. تم استخدام خمسة وثلاثون جرذاً من نوع ألبينو على النحو التالي : المجموعة (1) تغذت بنظام غذائي أساسي وتم تصنيفها على أنها مجموعة سلبية ، وتم حقن الباقي بالستربتوزوتوسين (STZ) ، وتغذى على وجبات تجريبية لمدة أربعة أسابيع بعد تأكيد الإصابة بمرض السكري (DM). الفئران المصابة تم تقسيمها إلى 4 مجموعات ، المجموعة (2) المجموعة الإيجابية التي حقنت بالستربتوزوتوسين فقط ؛ المجموعة (3) جرذان DM عولجت بـ 5% من الكستناء الحلو؛ المجموعة (4) جرذان DM عولجت بنسبة 10% من الكستناء الحلو ومجموعة (5) جرذان DM عولجت بنسبة 15% من الكستناء الحلو. في نهاية الاربعة اسابيع مدة التجربة ، تم جمع عينات الدم لتحديد نسبة الجلوكوز ، HB1C ، وحمض البوليك ، ومستويات الكرياتينين واليوريا ، ومالونديالدهيد (MDA) ، والسوبر اكسيداز داي ميونيز (SOD) ، ومستويات الجلوتاثيون (GSH). أوضحت النتائج أن الجرذان التي حقنت بالستربتوزوتوسين أظهرت إصابة بداء السكري (موجبة DM) وزيادة معنوية ( $P < 0.01$ ) في كلا من جلوكوز الدم ، ومالونديالدهيد ، اليوريا ، كرياتينين وحمض البوليك في الدم وانخفاض معنوي ( $P < 0.01$ ) في مستوى السوبر اكسيداز داي ميونيز (SOD) ، ومستويات الجلوتاثيون (GSH). وقد أظهرت الفئران المصابة التي عولجت بالكستناء الحلو انخفاضاً معنوياً في جلوكوز الدم ، ومالونديالدهيد ، واليوريا ، والكرياتينين وحمض البوليك في الدم ، وزيادة في مستوى السوبر اكسيداز داي ميونيز (SOD) ، ومستويات الجلوتاثيون (GSH). ونظراً لتأثير الكستناء على خفض نسبة الجلوكوز في الدم ، وتعزيز حالة مضادات الأكسدة والتأثيرات الوقائية للكلى في الفئران المصابة بداء السكري ، فإنه يمكن استخدامه في السيطرة على مرض السكري وتأثيراته الضارة. بينما كشفت نتائج التقييم الحسي أن الكستناء بنسبة 5% و 10% و 15% تم قبولها من قبل المحكمين بمستويات مختلفة.