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Cortisol Lowering Effect of *Cyperus Esculentus* in Normal Weight Male Human Subjects

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ABSTRACT

Tigernut contains active ingredients such as sterols, alkaloids, tannins, saponins, resins and vitamins E and C. The study investigated how the Tigernut could influence the human blood cortisol level in the normal weight male human subjects under resting condition. A total of forty (40) subjects were involved in the study. They were likewise non-habitual Tigernut chewers and aged 18-28 years were used for the study. Having taken the anthropometric data of the control subjects, they relaxed for the duration of 1hour 30 minutes. Cortisol hormone level was determined according to the method of Tiez and Andresen, using ELISA TECO kit for cortisol. In respect to the test subjects and on a different day entirely, 5g of the Tigernut was administered to each of them to be chewed as a bolus. It should be noteworthy that the same subjects used as control subjects were also used as the test subjects. After ingestion of the Tigernut, 50ml of water was given to each volunteer to flush the masticated Tigernut down the gut. The subjects were likewise allowed to rest for 1hour 30 minutes after which another blood cortisol level was taken. Statistical analyses were carried out using Graph Pad Prism Statistical Software Version 8.1. The results showed that the Tigernut significantly decreased ($P < 0.05$) the blood cortisol level after its consumption (from 13.36 ± 0.408 to 5.663 ± 0.139 µg/dl). Summarily, the Tigernut was observed to have a blood Cortisol lowering effect in the normal weight male human subjects.

Keywords: *Tigernut, cortisol level, sterols, alkaloids, tannins, saponins*

INTRODUCTION

Cyperus esculentus L., often known as chufa or earth almond, is a perennial tuberous plant from the sedge family (*Cyperaceae*). It is cultivated in Brazil, Spain, Africa, and Portugal. In Ukraine, earthnut is grown on experimental and household plots (Marchyshyn et al., 2018).[23] *Cyperus esculentus L.* is grown for its small, sweet, almond-shaped nodules, which are a source of edible oil. Tubers of earthnut formed at the roots have a hard shell and crispy flesh and a pleasant sweet taste. Chufa tubers have been used as food since ancient times due to their great nutritional value. They include: 20-25% fats, 20-35% starch, 12-28% carbohydrates, and 5-9% proteins. The oil is used actually in food, in the processing industry and in medicine. Tubers of earthnut are considered promising for the production of dietary, children's and special purposes, due to their content of biologically active substances (Marchyshyn *et al.*, 2018).[22,24] Its fat composition is similar to that of olive plant with 72% unsaturated (oleic acid and linoleic acid) and 28% saturated (palmitic

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acid and stearic acid) fatty acids (Ezeh *et al.*, 2014).[11]

Cyperus esculentus (CE) is also gluten and cholesterol free (Wills *et al.*, 1980).[36] It is the richest food source of flavonoids and also rich in water, fibers, alkaloids, digestible carbohydrates, saponins and fatty oils (glycerides). In addition, it also contains some elements, like phosphorus, potassium, calcium, iron, zinc, magnesium and manganese (Addy and Eteshola, 1984; Jeong *et al.*, 2000; Belewu and Belewu, 2007).[2,3,19] Cyperus esculentus oil is abundant in monounsaturated fatty acids, like olive, avocado, and hazelnut oil (Ezeh et al., 2014).[12] According to Sanchez-Zapata et al. (2012) and Ezeh et al. (2014), this monounsaturated oil contains large amounts of unsaponifiable matter, phospholipids, and bioactive components such tocopherols, phytosterols, and polyphenols. Previous research suggests that tigernut oil includes phytosterol, vitamin E, and β-carotene, which may contribute to its antioxidant effect (Hernández-Olivas et al., 2022). [16]

The hormone, cortisol, is otherwise known as stress hormone. Stress can lead to both physical and psychological health issues. Some stress can be useful at times, providing a boost that gives people the motivation and energy they need to get through situations like exams or work deadlines. However, excessive stress can have detrimental implications, affecting the immunological, cardiovascular, neuro-endocrine, and central neurological systems (Anderson, 1998).[26,27] Chronic stress, in particular, can have major consequences due to high amounts of chemicals released in the "fight or flight" response, which involves the endocrine system releasing glucocorticoids (Edwards, 2012; Whirledge and Cidlowski, 2013).[10,34] Cortisol, which is synthesized from cholesterol, is the main glucocorticoid in the zona fasciculata of human adrenal cortex. Its secretion in response to biochemical stress contributes to the well-characterized suppression of the hypothalamic-pituitary-adrenal (HPA) axis on health and cognition events (Oswald *et al.*, 2006; Juster *et al*., 2010).[20,28]

Some medicinal plants including tigernut (*Cyperus esculentus*) have been shown to have positive health effects (Fischer *et al.*, 1998; Lemaure *et al.*, 2007).[13,21] The yellow type is preferred above others due to its intrinsic characteristics such as huge size, appealing hue, and fleshier texture. It also produces more milk after extraction, has less fat and more protein, and includes less anti-nutritional elements, particularly polyphenols. The search for medicinal plants with a long history of use and small side effects is of interest to our society. The basic appointment of using plants is the control of metabolic disorders, as plant metabolites are close to the metabolites of the human body (Budniak *et al.*, 2021).[4]

Cortisol is released with a diurnal cycle and its release is increased in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system and to aid in the metabolism of fat, protein and carbohydrates. It also reduces bone development. Many of these tasks are performed by cortisol attaching to glucocorticoid or mineralocorticoid receptors within the cell, which subsequently bind to DNA and influence gene expression (Cole et al., 2019).[8]

In general, cortisol promotes gluconeogenesis (the synthesis of 'new' glucose from non-carbohydrate sources, which happens primarily in the liver but also in the kidneys and small intestine under specific conditions). The net effect is an increase in the concentration of glucose in the blood, further complemented by a decrease in the sensitivity of peripheral

tissue to insulin, thus preventing this tissue from taking the glucose from the blood. Cortisol has a permissive effect on the actions of hormones that increase glucose production, such as glucagon and adrenaline (Sabir *et al.,* 2019).[30]

Cortisol also plays a significant, albeit indirect, role in liver and muscle glycogenolysis (the breakdown of glycogen into glucose-1-phosphate and glucose), which is triggered by glucagon and adrenaline. Furthermore, cortisol promotes the activation of glycogen phosphorylase, which is required for adrenaline to influence glycogenolysis (Janah et al., 2019).[18] Cortisol, paradoxically, increases both gluconeogenesis and glycogenesis in the liver. Cortisol is thus better understood as promoting glucose/glycogen turnover in the liver. This contrasts with cortisol's impact in skeletal muscle, where glycogenolysis is indirectly stimulated by catecholamines (Hatting et al., 2018).[15]

Cortisol counteracts insulin, contributes to hyperglycemia by promoting gluconeogenesis, and inhibits the peripheral utilization of glucose (insulin resistance) by lowering the translocation of glucose transporters to the cell membrane (particularly GLUT4). Cortisol also increases glycogen synthesis (glycogenesis) in the liver, storing glucose in easily accessible form. The permissive effect of cortisol on insulin action in liver glycogenesis is observed in hepatocyte culture in the laboratory, although the mechanism for this is unknown (Dimitriadis *et al.,* 2021).[9]

Underlisted are some factors that can increase cortisol level: viral infections increase cortisol levels through activation of the HPA axis by cytokines (Petrescu *et al.,* 2018),[29] Intensive (high VO2 max) or extended aerobic exercise transiently elevates cortisol levels to enhance gluconeogenesis and sustain blood glucose; however, cortisol decreases to normal levels after eating (restoring a neutral energy balance). (Shetty, 2019.)[31,32] severe trauma or stressful events can elevate cortisol levels in the blood for prolonged periods (Chu *et al.,* 2021),[5] low-carbohydrate diets cause a short-term increase in resting cortisol (~3 weeks), and increase the cortisol response to aerobic exercise in the short- and long-term (Whittaker *and* Harris, 2022)[35] and increase in the concentration of ghrelin, the hunger stimulating hormone, increases levels of cortisol (Hanis *et al.,* 2020).[14]

Quite a numbers of researches have been carried out using medicinal plants to mitigate various disease conditions and the results have demonstrated that it provides efficient and cost-effective therapeutic solution and as such *Cyperus esculentus* oil is a potent nutraceutical though not totally new still remains largely unexploited and underutilized (Mohdaly, 2019).[25] The present study attempts to explore the effect of Tigernut on blood cortisol level in the normal weight male human subjects.

MATERIALS AND METHODS

Subjects

A total of forty (40) subjects were involved in the study (Oguwike *et al*., 2017).They were normal weight male volunteers. They were likewise non-habitual Tigernut chewers; aged 18-28 years were used for the study. Individuals from Ambrose Alli University were used and their health status was assessed with the aid of questionnaire and physical examination (Ugwu and Oyebola, 1996). Before beginning the trial, each subject provided informed consent. Prior to beginning the investigation, the University granted ethical permission.

Inclusion/Exclusion Criteria

Subjects with hypertension (Artfield, 1985), kidney and heart related conditions (Chukwu *et al*., 2006), those with ulcer,

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diabetes, any other health-related challenges, etc. were excluded from the study. Normal weight subjects (body mass index between 18.5-25.0 kg/m^2) were under-studied (Omorede *et al*., 2016). Before the study, the subject's age (years), weight (kg), height (m), body mass index (kg/m^2), systolic and diastolic blood pressure (mmHg) and heart rate (beats/min) were recorded.

Determination of Body Mass Index
Body mass index was calculated as the ratio of body weight in Kg / height in square meter. The subjects were asked to remove shoes and heavy clothes before measurement of weight and height. The balance (Camry, Italy) was used for measuring weight (in kilogram) and statiometer (Henglida, China) for measuring height (in centimeters, which was converted to meters). People with BMI between 18.5 and 24.9 were included in the study.

As regards the control subjects, they came into the Laboratory fasting (Marriot, 1993). They were made to relax before the commencement of the experiment. Having taken their anthropometric data, the subjects further relaxed for the duration of 1hour 30 mins (Igwe *et al*., 2007). Their blood samples were then taken for the determination of the control subjects' blood cortisol levels.

In respect to the test subjects and on a different day entirely, 5g of the *Tigernut* (Oguwike *et al*., 2017) was administered to each of them to be chewed as a bolus. (Igwe *et al*., 2007) It should be noteworthy that the same subjects used as control subjects were also used as the test subjects. After ingestion of the Tigernut, 50ml of water (Igwe *et al*., 2007) was given to each volunteer to flush the masticated *Tigernut* down the gut. The subjects were allowed to rest for 1h 30 minutes (Igwe *et al*., 2007) after which another blood samples were collected for the determination of the test subjects' blood cortisol levels.

Collection of Blood Sample
A 3ml blood sample was collected from medial cubital vein using vacutainer syringe on the same day that the serum sample was collected. Blood sample was transferred into an anticoagulant-free tube. After allowing for about 60 min for spontaneous blood clotting, the serum was separated by centrifugation at 3,000 rpm for 10 minutes at room temperature

Measurement of Cortisol
Cortisol hormone level was determined according to the method of Tiez and Andresen, (1986) using ELISA TECO kit for cortisol.

Principle
The cortisol ELISA Kit is a solid phase enzyme-linked immunosorbent test (ELISA) that uses competitive binding. The microtiter wells are coated with an antibody that targets a specific antigenic location on the cortisol molecule. Endogenous cortisol from a patient sample competes with a cortisol horseradish peroxidase compound for binding to the coated antibody. After incubation, the unbound conjugate is rinsed away. The amount of bound peroxidase conjugate is inversely related to the cortisol levels in the sample. The intensity of color formed after adding the substrate solution is inversely proportional to the cortisol levels in the patient's sample.

Assay Procedures
All samples and reagents were allowed to reach at room temperature ~25°C). Reagents were mixed by gentle inversion before use. Standards, controls and samples were assayed in duplicate.
1. Microtitration Strip was marked to be used.
2. Twenty-five µL of standards, controls, and samples were added to each well.

3. Two hundred µL of Conjugate Reagent were added into each well using a precision pipette.
4. The wells were mixed for 10 seconds.
5. The wells were incubated for 60 minute at room temperature (~25°C).
6. Each well was aspirated and washed 3 times by added 400 µL of working Wash Solution.
7. Two hundred µL of substrate solution were added into each well using a precision pipette and gently mixed for 10 seconds.
8. The wells were incubated in the dark for 15 minutes at room temperature (~25°C).
9. One hundred µL of Stop Solution were added into each well using a precision pipette and mixed for 10-20 seconds.
10. The absorbances of the solution in each well were read at 450 nm.

Calculation

The absorbance for each standard, control, or samples were obtained, and then the standard curve was prepared by plotting the absorbance readings for each of the standards along the Y-axis versus standard concentrations in ng/mL along the X-axis, the mean absorbance values for each sample were calculated using the relevant cortisol levels in ng/mL from the standard curve.

Statistical Analysis

Statistical analyses were carried out using Graph Pad Prism Statistical Software version 8.1. The results were presented as Mean ± SEM. A P-value of less than 0.05 was considered to be statistically significant

RESULTS

The Anthropometric Indices of Young Adults following an intake of Tigernut

Table 1 shows the anthropometric indices of young adults following an intake of tigernut. The Mean ± SEM of Age (years) was 22.71 ± 0.303, Height (m) was 1.776 ± 0.009, Weight (kg) was 67.29 ± 1.023 while BMI (kg/m^2) was 21.28 ± 0.295.

Parameters	**Mean ± SEM**
Age (years)	22.71 ± 0.303
Height (m)	1.776 ± 0.009
Weight (kg)	67.29 ± 1.023
BMI (kg/m^2)	21.28 ± 0.295

The effects of Tigernut consumption on cortisol level in young adult individuals

Table 2 shows the effects of Tiger nut consumption on cortisol level in young adult individuals, where cortisol (µg/dl) before intake had a value of 13.36 ± 0.408 while after intake had a value of 5.663 ± 0.139. A Statistically significant variation (P≤0.05) was observed.

Parameters	**Before intake**	**After intake**	**P-values**
Cortisol (µg/dl)	13.36 ± 0.408	5.663 ± 0.139	< 0.0001

P < 0.05 indicates significant difference

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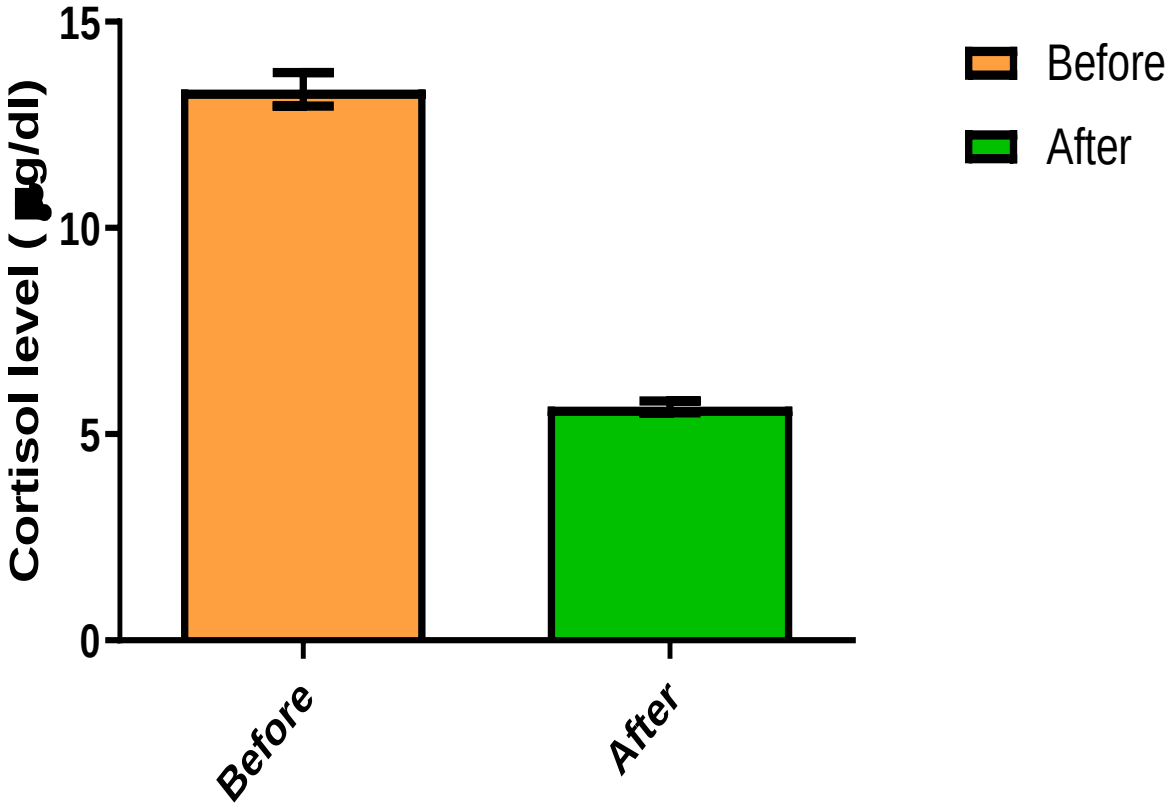


Fig. 1: *Showing the effect of Tigernut consumption on cortisol level in young adult individuals.*

There was a significant decrease after consumption of Tiger nut compared with before consumption

DISCUSSION

The study investigated how the Tigernut could influence the human blood cortisol level in the normal weight male human subjects under resting condition, and the possible mechanism(s) of action. The results showed that the Tigernut significantly decreased ($P < 0.05$) the blood cortisol level after its consumption (from 13.36 ± 0.408 to 5.663 ± 0.139 µg/dl).

Tigernut provides 77.49–80.01% necessary fatty acids and 31.32–34.03 mg/100 g essential amino acids (Ijarotimi et al., 2018). The tuber has a high concentration of the disaccharide D-saccharose, which when hydrolyzed yields D-glucose, D-galactose, D-xylose, and D-arabinose.[17]

The adrenal medulla secretes norephinephrine, which works with epinephrine to stimulate liver cells to release glucose to make more fuel available for cellular respiration. These hormones have short-term effects as the nerve impulses are sent from the hypothalamus. Hormones secreted by the adrenal cortex provide a slower, longer-acting (chronic) response to the stress. In this case, the hypothalamus secretes a releasing hormone, causing the anterior pituitary to secrete an adrenal-stimulating hormone called adrenocorticotrophic hormone (ACTH), which prompts adrenal cortex cells to make and emit corticosteroids.

Among them, mineralocorticoids, like aldosterone, can regulate water and sodium re-absorption in the kidneys. Glucocorticoids promote fat and protein breakdown and glucose synthesis. Cortisol (as a stress hormone) is the primary glucocorticoid that regulates or supports a range of critical cardiovascular, metabolic, immunologic, and homeostatic processes (Anderson, 1998).[1]

Quite interestingly, most of the studies on the impact of the tigernuts on the blood cortisol level would have been based on animal related experiments. This particular study is one of such few human related existing ones, if not the very pioneer study. And in comparison with some previous researches, which could be animal related, the findings are in agreement-in that the Tigernut contains a good quantity of vitamin B1, amongst others, which assists in balancing the central nervous system and helps to support the body to adapt to stress (Martinez, 2003). In the present study, the tigernuts significantly ($P < 0.05$) decreased the blood cortisol level after its consumption. Therefore, it could be recommended for combating stress in the human subjects.

Why the significant reduction in the blood cortisol level could be traceable to the fact that cortisol, which is synthesized from cholesterol, is the main glucocorticoid in the zona fasciculata of human adrenal cortex. Its secretion in response to biochemical stress contributes to the well-characterized suppression of the hypothalamic-pituitary-adrenal (HPA) axis on health and cognition events (Oswald *et al*., 2006; Juster *et al*., 2012). According to Belewu and Abodunrin (2006), tiger nut milk, unlike any other soft drink, is not only pleasant but also extremely healthful. It contributes to the reduction in the cholesterol by diminishing the 'bad' cholesterol low density Lipoprotein (LDL), and increasing the 'good' cholesterol, high density Lipoprotein (HDL). Its high content of vitamin E also collaborates against the cholesterol because it has an antioxidant effect over fats, which are ideal for coronary heart disease (Chukwuma *et al*., 2010).[6] Such a diminishing effect on cholesterol, which is a precursor in the production of cortisol could tremendously contribute to the reduction in blood cortisol level.

In addition, ascorbic acid presence (as in the tigernut), particularly in high doses had also been shown to mediate response to psychological stress and speed the decrease of the levels of circulating cortisol in the body post-stress. This could be indicated by a drop in systolic and diastolic blood pressures, as well as lower salivary cortisol levels after treatment with ascorbic acid (Ciji *and* Akhtar, 2021).[7]

Also noteworthy in the present study is the fact that low-carbohydrate diets could cause a short-term increase in resting cortisol (~3 weeks), and enhance the cortisol response to aerobic activity in both the short and long term (Whittaker and Harris, 2022). An rise in the concentration of ghrelin, the appetite inducing hormone, may increase the levels of cortisol (Hanis *et al*., 2020). It will interest you to know that the plant substance, tigernut, is already super rich in carbohydrate. This could be one of the factors whereby there was a significant ($P < 0.05$) decrease in the blood cortisol level. As regards the concentration of ghrelin, the hunger stimulating hormone, there would not have been an increase in blood cortisol level because the tiger nut itself is super rich in various forms of diet. This might have inhibited the hunger stimulating hormone; ghrelin which would have in turn significantly ($P < 0.05$) decreased the blood cortisol level.

Similar to the foregoing, before the release of cortisol by the Hypothalamo-Pituitary-Adrenal (HPA) Axis, the process of

stimulation of the HPA axis must have taken place (Van Slycke *et al.*, 2022).[33] But in this particular case study, the reverse was the case. This implied that the tigernut inhibited the said axis-with the resultant effect of a decrease in the blood cortisol level.

Summarily, the tigernut was observed to have a blood cortisol lowering effects in the normal weight male human subjects. Therefore, it could be recommended for an individual undergoing or known to have undergone stressful condition. The mechanisms involved could be multifactorial but a few of them have been speculated and enumerated above.

CONCLUSION

Conclusion, the tigernut was observed to have a blood cortisol lowering effects in the normal weight male human subjects. Therefore, it could be recommended for an individual undergoing or known to have undergone stressful condition. The mechanisms involved could be multifactorial but a few of them have been speculated and enumerated above.

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