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Effects Of CANDEREL® and The Ameliorative Role of Stem Cell Enhancer on Some Physiological Parameters in Male Albino Rats

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ABSTRACT

Background: CANDEREL® is non-nutritive artificial sweetener, which is mainly composed of aspartame and acesulfame potassium. **Materials and Methods:** thirty male albino rats weighing from 100 to 120 gm. The period of the experiment was 30 days. The animals were divided into three groups; **group 1:** control, **group 2:** rats received CANDEREL® (1 tablet/25kg b.w./day) and **group 3:** rats received CANDEREL® (1 tablet/25kg b.w./day) + SCE (9 mg/kg b.w./day). The following parameters were measured: serum glucose, ASAT, ALAT, serum creatinine, serum urea, protein and lipid profiles and hormonal levels (insulin, testosterone, serum T3 and serum T4). **Results:** there were many disturbances that occurred in the previous parameters, and SCE ameliorated most of these hazardous effects. **Conclusion**: artificial sweeteners are not safe in use; their disadvantages are more than their advantages. So, we recommended replacing non-nutritive sweeteners with nutritive ones to be away from any hazardous effects that may result from the use of artificial sweeteners. Also, SCE made a great job in fighting the impairments that occur during the experiment.

Keywords: CANDEREL®, aspartame, acesulfame potassium, stem cell enhancer, ASAT, ALAT, T3, T4, testosterone, insulin.

INTRODUCTION

Artificial sweeteners (ASs) have been introduced for more than a century as a way to provide a sweet taste of foods without the high energy content of thermal sugars. The consumption of artificial sweeteners has gained popularity largely because of low costs, low calorie and perceived health benefits to reduce weight and normalize blood sugar levels. For these reasons, artificial sweeteners are increasingly being introduced into commonly consumed foods such as diet soda, cereals and sugar-free sweets, and are recommended for weight loss and for individuals suffering from glucose intolerance and type 2 diabetes [(1)].

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a low calorie sweetener, which was discovered in 1965 and codified as E951. It is two hundred times sweeter than sucrose and readily dissolves in water [(2)]. Approximately 50% of the aspartame molecule is phenylalanine, 40% is aspartic acid, and 10% is methanol. Aspartic acid, a metabolite of aspartame, is an excitatory amino acid normally found in high levels in the brain. These levels are controlled by the blood-brain barrier, which protects the brain from large fluctuations in plasma aspartate [(3)]. Phenylalanine is an amino acid essential to the production of monoamine in the brain and is found in nearly all foods that contain protein. Due to high levels of phenylalanine in the blood, the consumption of aspartame may cause brain damage [(4)]. Among the metabolites, methanol, which is released during aspartame digestion, is a toxicant that causes systemic toxicity. Large doses of aspartame and its individual metabolites have been tested in humans and other animals. It has been reported that not only the metabolites of methanol but also methanol itself are toxic to the brain. The primary metabolic fate of methanol is direct oxidation to formaldehyde, then into formate. The toxic effects of methanol in humans are due to the accumulation of its metabolite formate and correlated with formate levels [(3)].

Acesulfame Potassium (acesulfame-K or ACK), is a non-nutritive artificial sweeteners, which is discovered in 1967. It is approximately 200 times sweeter than table sugar and codified as E950. Acesulfame potassium has been approved for use in a variety of food products including carbonated drinks, baking products, baby food and frozen food. Besides initiation of lingual sweet sensation, some studies have shown potential ACK-related genotoxicity effects and other cellular actions. Also, acesulfame potassium has been shown to induce insulin-secretion [(5)]. **Swithers *et al.*[(6)]** reported that male rats given an ACK yogurt diet gained more weight than rats fed with a glucose-sweetened yogurt diet. The potential link between the dietary use of artificial sweeteners and the generation of generic metabolic syndrome has not been convincingly investigated and thus still remains highly controversial [(5)].

CANDEREL® is made and used in Czech Republic. It contains aspartame and acesulfame potassium.

Stem cell enhancer (StemEnhance®) is a novel mobilizer of bone marrow adult stem cells that was shown to increase the number of circulating stem cells. One gram of StemEnhance® has shown to support an increased release in the number of circulating stem cells in the body by 25% to 30% that greatly increased the potential of the body's active repair and system renewal [(7)]. StemEnhance® is a blend of 4 compounds:

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Aphanizomenonflos-aquae (AFA) that extracted from blue-green algae, *Undaria pinnatifid, Polygonum multiform, and Cordyceps sinensis*. They may have an individual physiological effect or synergistic effects with one another [8]. Several studies have reported the ability of various types of stem cells to improve the pathological outcome of liver cirrhosis and to attenuate the clinical symptom of the disease [7].

The research questions arise here:

1- Does the use of both sweeteners aspartame and acesulfame potassium are more danger on human health than the use of only one of them?
2- Can stem cell enhancer ameliorate the effects of CANDEREL®?

So, this study aimed to clarify the dual effects of CANDEREL® and the protective role of stem cell enhancer.

AIM OF THE WORK

To illustrate the harms of CANDEREL® and the ameliorative role of stem cell enhancer (SCE) in male albino rats.

MATERIALS AND METHODS

CANDEREL®: consists of lactose, 8.5 mg of aspartame, 5.7 mg of acesulfame potassium, stabilizers [E460 (i), E468, E470 (a)], flavouring (maltol). It is manufactured in Czech Republic by Czech Manufacturing. CANDEREL® is a registered trademark of Merisant Company 2, Sarl.

Stem cell enhancer (StemEnhance®): STEM Tech Health Sciences, San Clemente, CA, USA (Stemtech TM).

Thirty male albino rats (weighing from 100 to120 g) were used in this study. Animals were housed in stainless steel cages, fed on rat chow and offered water *ad libitum*. The animals were divided into three equal groups (10 rats each) as follows: **first group:** the control untreated group, **second group:** rats received orally CANDEREL® (1 tablet/25kg b.w. /day),each tablet contains 8.5 mg of aspartame and 5.7 mg of acesulfame potassium and **third group:** rats received orally CANDEREL® (1 tablet/25kg b.w. /day) and stem cell enhancer (StemEnhance®) (9 mg/kg b.w./day). Body weights were recorded at the beginning and at the end of the experiment. After 30 days, animals were weighed, and then anaesthetized; blood samples were collected from retro-orbital vein, centrifuged for 15 min. at 5000 rpm. The clear non-haemolysed supernatant sera were quickly removed and then used for further biochemical analysis.

Biochemical Examination:

In the present study, total protein (TP) and albumin concentrations were estimated. Then, serum globulin concentrations were calculated according to the formula:

Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, creatinine, urea, glucose concentrations as well as lipid profile including total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. All parameters were estimated using **BioMerieux SA kits, France**.

The ratio of serum albumin/globulin was determined. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low-density lipoprotein cholesterol) using the **Friedwald's [9]** and **Norbert [10]** formulas, respectively as following:

Friedewald's [9] equation: **LDL (mg/dl) = TC- {HDL + [TG/5]}**.

Norbert [10] equation: VLDL = TG/5

Insulin was determined using **ELISA kit** (10-1250-01, Mercodia AB, Uppsala, Sweden). **The HOMA-2 IR index** (**Homeostatic Model Assessment of Insulin Resistance**) was calculated by a free online calculator (HOMA Calculator, Version 2.2.3, Diabetes Trail Unit, The University of Oxford, Oxford, UK).

$$\text{HOMA-IR} = \frac{[(\text{Glycaemia}(\text{ mg/dl})/18.2) \times \text{Insulin (mU/ml)}]}{22.517} \quad [11]$$

Testosterone, T3 and T4 were estimated by using **VIDAS® kits**, which are automated quantitative tests.

Ethical Approval:

This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of science, Al-Azhar University, Cairo, Egypt. The study was approved by the Ethics Board of Al-Azhar University.

Statistical analysis

The results were expressed as mean ± SE of the mean. Data were analyzed by using t-test and performed using the Statistical Package (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance between groups.

RESULTS

As regards body weight, significant increase ($p < 0.05$) was noticed in the percentage of body weight change in CANDEREL® group, while SCE-treated group recorded no significant change as compared to control animals (Table 1).

In regard to glucose level, insulin level, and HOMA-IR, there was significant increase ($p < 0.05$) in their levels in CANDEREL® group, meanwhile, SCE-treated group revealed no significant change in glucose level and HOMA-IR, but there was significant increase ($p < 0.05$) in insulin level in contrast with control rats (Table 1).

Table (1): Percentage of body weight change, glucose level, insulin level and HOMA-IR in control, CANDEREL®- or CANDEREL®+SCE-treated animals

Groups / Parameters	Control	CANDEREL®	CANDEREL®+SCE
% of body weight Change	8.6 ± 0.5	13 ± 1.28*	9.7 ± 0.7
% of change from control		51%	13%
Glucose (mg/dl)	109 ± 0.7	115 ± 1.66*	111 ± 1.2
% of change from control		5%	2%
Insulin (mlU/ml)	0.97 ± 0.007	1.4 ± 0.13*	1.2 ± 0.071*
% of change from control		44%	24%
HOMA-IR	0.26 ± 0.003	0.39 ± 0.039*	0.325 ± 0.029
% of change from control		50%	25%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

As regards protein profile, animals that received CANDEREL® had significant decrease (p <0.05) in serum total protein, albumin and albumin/globulin ratio, while those received SCE showed no significant change in protein profile parameters as compared to the corresponding control group (Table 2).

Table (2): Serum total protein, albumin, globulin and albumin/globulin ratio in control, CANDEREL®- or CANDEREL®+SCE-treated animals.

Groups / parameters	Control	CANDEREL®	CANDEREL®+SCE
Total Protein (g/dl)	6.63 ± 0.04	5.66 ± 0.34*	6.4 ± 0.11
% of change from control		-15%	-3%
Albumin (g/dl)	3.43 ± 0.046	2.53 ± 0.3*	3.24 ± 0.13
% of change from control		-26%	-6%
Globulin (g/dl)	3.2 ± 0.028	3.13 ± 0.026	3.16 ± 0.024
% of change from control		-2%	-1%
Albumin/Globulin	1.074 ± 0.02	0.81 ± 0.077*	1.02 ± 0.04
% of change from control		-25%	-5%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

Concerning liver functions, ASAT and ALAT activities revealed significant increase (p <0.05) among CANDEREL® group and no significant change among SCE-treated group in contrast with control rats (Table 3).

Table (3): ASAT and ALAT activities in control, CANDEREL®- or CANDEREL®+SCE-treated animals

Groups / parameters	Control	CANDEREL®	CANDEREL®+SCE
ASAT (U/L)	115.88 ± 0.3	123 ± 2.25*	117 ± 1.3
% of change from control		6%	1%
ALAT (U/L)	55.4 ± 0.16	62 ± 1.97*	56.5 ± 0.8
% of change from control		12%	2%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

Regarding lipid profile, the present results revealed significant increase (p <0.05) in total cholesterol, triglycerides and ratios of TC/HDL-C (risk factor 1) and LDL-C/HDL-C (risk factor 2) and highly significant increase (p < 0.01) in LDL-C. On the other hand, there was significant decrease (p < 0.05) in HDL-C, and no significant change was found in VLDL-C in rats received CANDEREL® as compared to control group. Meanwhile, there was no significant change among lipid profile in rats received SCE (Table 4).

Table (4): Changes in total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, VLDL-C, TC/HDL ratio and LDL/HDL ratio in control, CANDEREL®- or CANDEREL®+ SCE-treated animals

Groups / parameters	Control	CANDEREL®	CANDEREL®+SCE
Total Cholesterol (mg/dl)	121.1 ± 0.38	130 ± 2.68*	123 ± 1.8
% of change from control		7%	2%
Triglycerides (mg/dl)	135 ± 0.7	144 ± 2.63*	137 ± 1.7
% of change from control		7%	1%
HDL-C (mg/dl)	59.4 ± 0.48	52 ± 2.17*	58 ± 1.3
% of change from control		-12%	-2%
LDL-C (mg/dl)	34.6 ± 0.48	49.2 ± 2.4**	37.6 ± 1.5
% of change from control		42%	9%
VLDL (mg/dl)	27 ± 0.1	28.8 ± 1.1	27.4 ± 0.4
% of change from control		7%	1%
TC/HDL	2.04 ± 0.015	2.5 ± 0.15*	2.12 ± 0.06
% of change from control		23%	4%
LDL/HDL	0.6 ± 0.01	0.94 ± 0.12*	0.65 ± 0.05
% of change from control		56.6%	8%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

Concerning kidney functions, serum urea and creatinine showed significant increase (p <0.05) in CANDEREL® group, while there was no detectable change in kidney functions in SCE-treated group as compared to control animals (Table 5).

Table (5): Serum urea and creatinine levels in control, CANDEREL®- or CANDEREL®+SCE-treated animals

Groups / parameters	Control	CANDEREL®	CANDEREL®+SCE
Urea (mg/dl)	37.3 ± 0.66	43.8± 1.85*	38± 0.7
% of change from control		17%	2%
Creatinine (mg/dl)	1.18 ± 0.025	1.67± 0.15*	1.22± 0.04
% of change from control		41.5%	3%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

In relation to hormones, CANDEREL® group revealed significant decrease (p <0.05) in the levels of testosterone, T3 and T4 hormones but no detectable change was found in their levels in SCE-treated group as compared to control rats (Table 6).

Table (6): Serum testosterone, T3 and T4 levels in control, CANDEREL®- or CANDEREL®+SCE-treated animals.

Groups / parameters	Control	CANDEREL®	CANDEREL®+SCE
Testosterone (ng/dl)	1.03 ± 0.004	0.89 ± 0.043*	0.99 ± 0.02
% of change from control		-14%	-4%
T3 (ng/dl)	47.9 ± 0.6	42.5 ± 1.6*	46 ± 0.9
% of change from control		-11%	-4%
T4 (µg/dl)	3.036 ± 0.009	2.3 ± 0.23*	2.88 ± 0.11
% of change from control		-24%	-5%

Values represent mean ± SE (standard error). (*: p<0.05, **: P< 0.01 as compared to control group)

DISCUSSION

In this study, CANDEREL® revealed an increase in the percentage of body weight change, which may be due to the effect of aspartame. Ingestion of aspartame results in a craving for carbohydrates, which eventually result in weight gain, especially because the formaldehyde stores in the fat cells, particularly in the hips and thighs; therefore, aspartame is believed to cause problem in diabetic control [(12)].

Also, aspartame use significantly decreases the concentrations of leptin in plasma. Leptin acts on the brain by inhibiting food intake, but lower concentrations of leptin could stimulate appetite [(13)]. **Feijo *et al.*** [(14)] reported that there was an increase in body weight and fluid intake in a group of rats treated with aspartame. By using stem cell enhancer, there was amelioration occur to the percentage of body weight change.

There was an increase in glucose level, insulin level and HOMA-IR in rats received CANDEREL® compared to the control group. The exposure to artificial sweeteners including aspartame, promote impairment in insulin sensitivity [(15)]. Moreover, it was indicated that phenylalanine, a metabolite of aspartame, might stimulate an increase in insulin and glucagon levels in serum of healthy individuals and induce an elevation of hepatic glucose production and its level in blood of rats [(16)]. On the other hand, treating with stem cell enhancer resulted in amelioration of glucose level and HOMA-IR with an increase in insulin level. This could possibly be due to the high fiber content of blue-green algae that interferes with the glucose absorption or probable action of producing polypeptides after digestion of blue green algae [(17)]. The results of this study are in agreement with the results of **Sanaei *et al.*** [(18)] and **Anwer *et al.*** [(19)] who recommended the use of StemEnhance® as a functional food in management of diabetes. They attributed the antihyperglycemic effects of StemEnhance® to increase the insulin secretion from β-cells of the pancreatic islet or due to enhancement of transport of blood glucose to the peripheral tissue.

In the present work, there was a reduction in the levels of total protein, albumin and albumin/globulin ratio in rats received CANDEREL®. These results were in accordance with **Helal *et al.*** [(20)] who announced an inhibitory effect of artificial sweeteners on the biosynthesis of protein and albumin. These reductions indicate hepatotoxicity and liver damage that led to inability of liver to produce proteins and causing utilization of amino acids for the oxidation or gluconeogenesis. Meanwhile, the use of SCE made a great enhancement in all protein profile parameters in this study. This may be due to the antioxidant effect of *Undaria pinnatifida*, which is one of the components of SCE; it has a potential antioxidant that can effectively abrogate oxidative stress [(21)].

There was an increase in liver functions in rats received CANDEREL®. The increase of these biomarkers levels indicates an enhanced permeability, tissue damage or necrosis due to formation of free radicals in all tissues from glucose auto-oxidation and protein glycosylation [(22)]. Also, possibly this may be attributed to methanol, the byproduct from aspartame metabolism, which is reported to produce altered oxidant/ antioxidant balance and surface charge density causing the leakage of ALAT and ASAT. Free radical attack unsaturated fatty acids in the cellular membrane resulting in membrane lipid peroxidation, which decreases its fluidity leakage of enzymes and loss of receptor activity as well as damage of membrane proteins leading to cell inactivation [(16)]. On the other hand, by using SCE, liver functions returns back close to normal. This may be due to the antioxidant activity of some StemEnhance® constituents that exhibited a hepatoprotective effect against liver damage caused by these sweeteners [(7)].

In the present work, there was an increment in lipid profile parameters except HDL-C which revealed a reduction in its level in CANDEREL®-treated rats. Chronic exposure to aspartame induced changes in biochemical parameters and lipid metabolism, and could be involved in the development of hyperglycemia and hypercholesterolemia. Other authors have shown that the long-term consumption of artificial sweeteners, such as aspartame, might contribute to hypersensitivity reactions and atherosclerosis development [(23)]. Hypercholesterolemic atherosclerosis was associated with increases in ROS, which represents a critical initiating event in the development of atherosclerosis and cardiovascular diseases. Oxidative stress and oxidative damage of tissue could be the initial markers of some chronic diseases, like diabetes. During diabetes, persistent hyperglycemia causes the increased production of free radicals in all tissues from glucose auto-oxidation and protein glycosylation [(24)]. By using SCE, there was an enhancement in lipid profile levels. Some studies showed that blue-green algae inhibits intestinal cholesterol absorption and decreases the hepatic lipids and leads to attenuation of plasma total cholesterol and triglyceride concentrations. Blue-green algae can also decrease oxidative stress due to their free radical scavenging activity and inhibition of lipid per-oxidation [(25)].

There was an increase in kidney functions in CANDEREL®-treated rats. The elevated levels of urea and creatinine are indicator for renal dysfunction. This may be due to the oxidative stress, which is caused by treatment with aspartame [(26)]. When liver and kidney cell membrane is damaged due to involvement of oxidative stress during aspartame metabolism, a variety of enzymes normally located in the cytosol is released into the blood stream [(27)]. By using SCE, urea and creatinine returns back close to their normal

values. This may be due to *Spirulina platensis*, which significantly decreased the elevated levels of creatinine and urea. It may be possible, that *Spirulina*, due to its potential antioxidant properties, improved renal function via attenuating oxidative stress-mediated decline in kidney. The nephroprotective effects of *Spirulina* have been reported against renal injury induced by gentamicin [(28)].

There was a reduction noticed in testosterone hormone in CANDEREL® group. Aspartame could be responsible for reduction in testosterone hormone followed by decrease in acetyl cholinesterase enzyme activity [(29)]. Aspartame is called excitotoxin. It has the ability to bind to different brain receptors, causing them to take in too many calcium ions and eventually causing damage. Unfortunately, these negative effects can not only impact the brain but testosterone production as well [(30)]. By using SCE, there was amelioration in testosterone hormone. This may be due to the antioxidant properties of *Spirulina platensis*, which contain potent antioxidant components as beta-carotene, vitamin C, vitamin E, selenium, and manganese. The antioxidants found in *Spirulina platensis* reduce oxidative stress and relieve the pathological changes induced by CANDEREL® in testis, which may lead to improvement of testosterone level [(31)].

Another reduction was found in T3 and T4 hormones in CANDEREL® group. A study done on male albino rats showed that formaldehyde (a metabolite of aspartame) causes the regression of the follicular epithelial cells of the thyroid gland, which leads to decreased levels of T3 and T4, and increased TSH levels [(32)]. Also, it was reported that the use of aspartame may be linked to the development of a type of hypothyroidism called Hashimoto's thyroid disease (HT) [(33)]. On the other hand, SCE caused enhancement in thyroid hormones. *Cordyceps sinensis* has been reported to have immunomodulatory effects on many autoimmune-inflammatory diseases including cerebral disease (in rat), type 1 diabetes, asthma, chronic obstructive pulmonary disease and chronic nephritic disease [(34)]. In China, some recent studies reported that *Cordyceps sinensis* could significantly reduce serum titer of anti-thyroid autoantibody in both Graves' disease (GD) and Hashimoto's thyroiditis (HT). Moreover, it was verified that *Cordyceps sinensis* could be effective on cell-mediated immunity in animal models of many diseases [(35)].

From the previous results, we can notice that SCE increased thyroid hormones (up to normal levels), which leads to a reduction in body weight as compared to CANDEREL® group.

RECOMMENDATION

Although many people are starting to use artificial sweeteners and sugar substitutes, we believe that it is best to stick with natural sugar. Despite the fact that white table sugar, or sucrose, has been processed and bleached, it is very close to natural compared to other sweeteners. Humans are consuming white sugar for a very long time and it seems to have few problems.

CONCLUSION

In our study, CANDEREL® made a lot of disturbances in most biological systems, while stem cell enhancer ameliorated most of it.

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