Advances in Dairy Research

OPEN ACCESS Freely available online

Research Article

Evaluation of the Antidepressant-Like Activity of Ice Cream Containing Some Milk Derived Proteinous Substances in Mice

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ABSTRACT

The aim is to behaviorally and biochemically evaluate the antidepressant- like effect of vanilla ice cream in mice, with the light on the physicochemical quality of the resultant ice cream functional product. Whey protein isolate, lactoferrin or α-casozepine was applied in ice cream as milk derived neurogenesis precursors at a rate of 3% with curcumin or β-carotene as phytochemical antidepressant colouring agent at a rate of 0.3%. Male albino mice were used for the evaluation; soy protein-based diet was introduced as standard, and tramadol was administered as anti-depression reference. The results indicated that the functional protein ingredients led to increase overrun and food efficiency ratio (FER), and enhance the melt resistance, and to decrease the values of ash and lactose. The curcumin led to decrease the FER, while β-carotene led to decrease γ-aminobutyric acid. Lactoferrin caused increments in the FER and swimming time as well as decreasing the immobility time, whereas α-casozepine decreased both immobility time and γ-aminobutyric acid level. Furthermore the combination of curcumin and α-casozepine raised serotonin,epinephrine and glutamate levels, besides decreasing the immobility time.

Keywords: Lactoferrin; β-carotene; Despair

INTRODUCTION

Ice cream is one of the most widely consumed delicious and nutritious dairy products whole over the world. Recently, the concern of researches has been focused on such rich popular foods which can be vehicles for many functional aspects with effective roles in controlling specific disorders [1]. The WHO indicated that greater than 121 millions of people suffer from depression with its harms on economy and life style [2]. The most dominating physiological cause of depression is the abnormal monoamine neuronal function; as a result of depletion of those monoamines, namely serotonin, norepinephrine and dopamine in the brain, so the antidepressant drugs were taken to increase the levels of the mentioned monoamines [3]. The majority of the antidepressant medications had adverse effects and complications such as fatigue, apathy, sleep disturbance, sexual dysfunction and cognitive impairment [4] so many researchers studied the antidepressant-like effects of the natural bioactive substances. Milk derived proteins and active peptides had proven ability to act as an antihypertensive, antitumor and antidepressant agent, due to their comparatively high contents of tryptophan, phenylalanine, tyrosine and glutamine which play a key role in the synthesis of the neurotransmitters [5]. Ahmed et al., [6] studied the antidepressant-like effect of whey protein isolate in a chronic unpredictable stress model in mice besides fluoxetine as reference and found that the whey proteins increased the glutamate and monoamines brain levels as well as inhibited the γ-aminobutyric acid release. Vekovischeva et al., [7] compared the effects of native whey protein and α-lactalbumin with casein on the behavior of mice; and found that introducing α-lactalbumin with diet showed anxiolytic and antidepressive effects whereas the native whey proteins enhanced sociability. Dhingra and Bansal [8] concluded that beta-carotene had antidepressant-like efficacy in stressed and unstressed mice throughout the antioxidant activity and decreasing the plasma nitrites and corticosterone contents. Nevertheless, most of the foregoing studies lacked to cover the application of the bioactive substances in a commercial food product with their technological functional properties, and considered those materials as pharmacological food supplements. For that in view, the present study was conducted to evaluate the antidepressant- like effect of the functional ice cream containing whey protein isolate, lactoferrin or α-casozepine as milk derived neurogenesis precursors, with curcumin or β-carotene as phytochemical antidepressant colouring agent, with the light on the effects of those ingredients on the physicochemical aspects of the final ice cream products.

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Received: December 31, 2020, **Accepted:** March 12, 2021, **Published:** March 19, 2021

Citation: Emam AO, El-Nashar HAS (2020) Evaluation of the Antidepressant-Like Activity of Ice Cream Containing Some Milk Derived Proteinous Substances in Mice. J Adv Dairy Res. 9: 255.

MATERIALS AND METHODS

Materials

Skimmed milk powder was obtained from Synlait Milk Ltd., Rakaia 7783 - New Zealand (3.8% moisture, 34% protein, 1.5% fat and 8.3% ash). Cow's fresh cream (56.5% fat, 0.371% ash and 1.71% protein) and cow's whole milk (3.5% fat, 0.696% ash and 2.89% protein) were obtained from Shahencoland Company for dairy products Foah, Egypt. Curcumin and β-carotene powders were obtained from Shaanxi Zhengsheng Kangyuan Biomedical Co., Ltd, Shaanxi, P.R. China. Carboxy methyle cellulose was obtained from modern beverage company for food industries "Solo", El-obour city, Egypt. Vanilla and sucrose were purchased from the local market of Egypt. Instant whey protein isolate was obtained from LACTALIS Ingredients Les Placis - 35230 Bourgbarré – FRANCE. (88.14% protein, 1.4% fat, 2.510% ash and 5.141% moisture). Lactoferrin was obtained from Fonterra brands, Ltd – Newzeland (96.14% protein, 0.6% ash and 3.43% moisture). α-casozepine was obtained from Ingredia dairy experts company, France (94.76% protein). Tramadol was obtained from Janssen Pharmaceuticals, Inc., USA.

EXPERIMENTAL PROCEDURE

Manufacturing procedures of Ice cream

The procedure of Akin et al., [9] was applied with modifications as a basic mix was formulated to contain 15% sucrose, 10% fat and 12% milk solids not fat. The dry ingredients (including; skim milk powder, sucrose and carboxy methyle cellulose) were mixed together. While whole milk and cream were transferred to the batch pasteurizer and warmed to 55°C then the mixed dry ingredients were added with stirring. The mix was then pasteurized at 85°C for 5 min then cooled to 50°C and divided into two equal portions the first one was for the curcumin containing treatments as curcumin powder was added at a rate of 0.3% and stirred well, the second portion was the β-carotene containing treatments as β-carotene powder was added at a rate of 0.3% and stirred well. Each portion was divided into three equal sub portions. Whey protein isolate, lactoferrin or α-casozepine was added to the sub portions at a rate of 3%. Mixes were homogenized separately at 50°C by using a pressure of 3000 PSI at first stage and 500 PSIat the second stage. Vanilla was added at a rate of 0.17%. Mixes were cooled to 4°C and frozen individually using a softy ice cream freezer (Technogel HMT®). The ice cream was collected in 500 mL plastic containers and kept frozen at -18 ± 1°C. Control was made from a conventional ice cream mix containing 15% sucrose, 10% fat and 15% milk solids not fat.

Analytical methods

Dry matter, fat and total nitrogen contents as well as titratable acidity were determined according to [10]. pH was measured using a 315i/SET pH-meter with a Sentix 42 electrode (WTW) after a 10-g sample was dissolved in 100 mL of distilled water, standard buffer solutions (pH 4.01 and 7.01; WTW) were used for calibration. Gravimetric overrun, first drip time and melting rate were determined as described by Whelan et al., [11].

For the biological evaluation, the ice cream samples were dried at 50°C in a vacuum oven. The biological experiments were carried out using standardized 48 Swiss male albino mice with the initial body weights ranging from 20 - 23 g and their age at the start of the study was 6 weeks. The animals were housed in individual metabolic cages. The cages were similar in construction to that described by Schiller [12] at a room of 25°C and 65% relative humidity, with a 12-h light-dark cycle. The animals were divided into 8 equal groups. Each group of mice was given separate diet for a period of 6 weeks as follows:

Group-1: This control group; was given standard soy protein-based diet *ad-libtum* according to the AIN-93M diet [13].

Group-2: This was given the standard soy protein-based diet *ad-libtum* and treated with 40 mg/kg/day of tramadol during the 6th week of the study.

Group-3: This was given 1 g/mouse/day of dried ice cream containing whey protein isolate and curcumin, then standard diet*ad-libtum*.

Group-4: This was given 1 g/mouse/day of dried ice cream containing lactoferrin and curcumin, then standard diet *ad-libtum*.

Group-5: This was given 1 g/mouse/day of dried ice cream containing α-casozepine and curcumin, then standard diet *ad-libtum*.

Group-6: This was given 1 g/mouse/day of dried ice cream containing whey protein isolate and β-carotene, then standard diet *ad-libtum*.

Group-7: This was given 1 g/mouse/day of dried ice cream containing lactoferrin and β-carotene, then standard diet *ad-libtum*.

Group-8: This was given 1 g/mouse/day of dried ice cream containing α-casozepine and β-carotene, then standard diet *ad-libtum*.

Mice's tail suspension test

Depending on that suspending mouse upside down leads to a characteristic behavior of immobility after initial temporary struggle. This behavior reflects a state of despair which can be moderated by several agents which are therapeutically effective in human depression. At the nights of days 30 and 35 of the experiment, tail suspension test was conducted and the average immobility time was recorded during a 6 min period as described by Rodrigues et al. [14].

Jar forced swim test

Mice were individually forced to swim for 5 min inside a vertical glass cylinder of 40 cm height and 15 cm diameter; containing 15 cm height of water maintained at 25 ± 2°C. Mice placed in the cylinder for the first moments were highly active, vigorously swimming in circles, trying to climb the wall then their activity began to subside and was interspersed with phases of immobility or floating of increasing length. A mouse was considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface, with no additional activity. The forced swim test was performed at 37th day and the duration of swimming and climbing was recorded as described by Porsolt et al. [15].

Food intake (FI) of each mouse was recorded daily. At the end of the 6th week body weight gain (BWG) and total FI were recorded. Food efficiency ratio (FER) for each group was calculated as following: FER = BWG/Total FI. The following tests were carried out to evaluate the antidepressant-like activity of the experimental functional ice cream samples.

Determination of brain γ-aminobutyric acid (GABA), glutamate, serotonin, and epinephrine and dopamine contents

On the forty second day mice were killed by decapitation. Hypothalamus parts were isolated and washed separately with 5°C physiological saline, blotted between two damp filter papers and stored at −80°C until use. Each hypothalamus tissue was weighed and homogenized in 75% aqueous HPLC grade methanol. The homogenate was spun at 4000 r.p.m. for 10 min and the supernatant was divided into two equal portions; the first was dried at room temperature and used for GABA and glutamate determination, whereas the second portion was used for monoamine determination. The precolumn PTC derivatization technique of HPLC was conducted for the determination of glutamate and GABA as described by Heinrikson and Meredith [16]. Hypothalamus monoamines were determined according to method described by Pagel et al., [17].

Statistical Analysis

The obtained results were statistically analyzed through the ANOVA procedure followed by Duncan's multiple range tests according to statistical analyses system user's guide [18]. Design was completely random with replications.

RESULTS AND DISCUSSION

Physicochemical properties of the functional ice cream

Data shown in Table 1 revealed that, the source of the phytochemical pigment had no significant effect on all the physicochemical determined criteria except a slight increase was observed in the protein and lactose values of the curcumin containing ice cream samples, and an increased meltability in the β-carotene containing samples. The type of functional protein had no significant effect on the total solids of the resultant functional ice cream. That could be due to the similar dry matter contents of the added functional protein sources. Likewise the type of the functional protein did not cause any significant differences in the ice cream fat and titratable acidity contents. The addition of lactoferrin to ice cream led to obtain the highest protein levels in the final product in comparison with the other functional protein types, while the α-casozepine ice cream samples came in the second order followed by the whey protein isolate (WPI) ones then the control which obtained the lowest protein level of all samples. That could be ascribed essentially to the comparatively high protein content of the used lactoferrin product. Concerning the ash content, the lactoferrin ice cream was associated with the lowest ash levels, followed by the WPI and α-casozepine ice cream samples which had statistical similar ash contents to each other's, while the control ice cream was distinguished with the highest ash of all samples. Regarding the lactose levels, the application of the functional protein ingredients led to reduce the lactose levels in the ice cream when compared to the control one. Amongst the functional ice cream samples, the WPI ice cream obtained the highest lactose level, while both the lactoferrin and α-casozepine ice cream samples contained statistically similar lactose contents and came in the latest order. That could be due to the relatively high lactose content of the whey protein isolate when compared to the other functional protein ingredients. This phenomenon is in accordance with that reported by Foegeding et al., [19] who

Table 1: Ls means values for phytochemical pigment source, type of functional protein and phytochemical pigment source × type of functional proteinon the physicochemical properties of functional ice cream.

Source of variation	Total solids (%)	Protein (%)	Fat (%)	Ash (%)	Lactose (%)	Titratable acidity (%)	Calcium (%)	Overrun (%)	First drip time (min)	Melting rate (g/min)
Source of the phytochemical pigment (SP)										
Curcumin	40.28^{a}	5.988^{a}	9.95^{a}	1.0253^{a}	6.139^{a}	0.190^{a}	0.1506^{a}	33.08^{a}	6.89^{b}	1.62^{b}
β-carotene	40.24^{a}	5.870^{b}	9.92^{a}	1.0222^{a}	5.802^{b}	0.187^{a}	0.1507^{a}	33.08^{a}	7.93^{a}	1.80^{a}
P-value	0.4825	0.0372	0.5489	0.8527	<.0001	0.6739	0.9731	1.0000	<.0001	<.0001
Type of functional protein (TF)										
Control	40.01^{b}	4.676^{c}	9.95^{a}	1.1618^{a}	7.075^{a}	0.180^{a}	0.1684^{a}	28.38^{d}	5.68^{d}	2.10^{a}
WPI	40.92^{a}	6.284^{b}	9.90^{a}	0.9883^{b}	5.813^{b}	0.189^{a}	0.1519^{b}	38.26^{a}	6.43^{c}	1.63^{c}
Lactoferrin	40.06^{b}	6.540^{a}	9.95^{a}	0.9273^{c}	5.484^{c}	0.199^{a}	0.1394^{c}	35.53^{b}	8.66^{b}	1.36^{d}
α-casozepine	40.06^{b}	6.216^{b}	9.95^{a}	1.0177^{b}	5.510^{c}	0.186^{a}	0.1428^{c}	30.16^{c}	8.88^{a}	1.76^{b}
P-value	<.0001	<.0001	0.7722	<.0001	<.0001	0.4372	<.0001	<.0001	<.0001	<.0001
TP × TF										
Curcumin × Control	40.04	4.736	10.10	1.1633	7.455	0.181	0.1693	29.87	5.45	1.93
Curcumin × WPI	40.93	6.356	9.90	0.9893	5.875	0.192	0.1513	38.03	6.03	1.51
Curcumin × Lactoferrin	40.07	6.596	9.90	0.9293	5.657	0.201	0.1393	36.32	7.57	1.34
Curcumin × α-casozepine	40.08	6.266	9.90	1.0193	5.572	0.188	0.1423	28.12	8.54	1.71
β-carotene × Control	39.99	4.616	9.80	1.1603	6.695	0.180	0.1674	30.45	5.92	2.28
β-carotene × WPI	40.90	6.213	9.90	0.9873	5.752	0.186	0.1524	38.49	6.83	1.75
β-carotene × Lactoferrin	40.05	6.485	10.00	0.9253	5.312	0.198	0.1395	34.75	9.76	1.38
β-carotene × α-casozepine	40.03	6.166	10.00	1.0160	5.449	0.184	0.1433	28.65	9.23	1.82
P-value	0.9958	0.9925	0.0094	1.0000	<.0001	0.9971	0.9486	<.0001	<.0001	0.0003

reported the whey protein isolates contains a combination of lactose, fat and ash at considerable levels ranging from 4 to 6%. The calcium contents of the functional ice cream took the same trends of lactose towards the type of the functional protein, as the control gained the highest calcium percentage followed by the WPI ice cream then both the lactoferrin and α-casozepine ice cream samples. That could be ascribed essentially to that the main source of the non-fat milk relatively high ash content, moreover the purification processes like solids in the control ice cream was the skim milk powder with its filtration and demineralization occurred to the used functional substances. With respect to the overrun, the application of the functional ingredients led to increase the overrun of resultant the ice cream possibly due to the increase in the protein contents which in turn enhance viscosity and limit the mobility of free water. Similar observation was reported by Sofian and Hartel [20] who found a positive correlation between the protein content of the ice cream mixes and their overrun. Amongst the experimental ice cream samples, the WPI ice cream was distinguished with the highest overrun as a result of retaining the maximum air during freezing and forming firm air cells able to prevent the shrinkage. These findings are in accordance with the observations of Syed et al., [21] who reported that foams resulted from whey protein had comparatively high yield stress and overrun. The application of the functional proteins significantly increased the first drip time. The highest first drip time was in the α-casozepine ice cream samples. Amongst the functional ice cream samples, the WPI ice cream was associated with the lowest first drip time. That could be due to the higher lactose content of the WPI ice cream than that of both lactferrin and α-casozepine samples, which in turn lowered the ice cream freezing point and hence increased the first drip time. Moreover, a negative correlation was observed between the melting rates of all ice cream samples and their protein contents, possibly due to the role of protein in raising viscosity and reducing the heat transfer rate through the ice cream [22]. For that in view, the application of the functional protein ingredients helped in enhancing the melting resistance of the ice cream.

Food efficiency ratio of the functional ice cream

Data of Table 2 demonstrated that, although the adjustment of the ice cream-feeding quantity at 1 g/mouse/day, the source of the used phytochemical pigment whether curcumin or β-carotene as well as the type of the applied functional protein whether the whey proteinisolate, lactoferrin or even α-casozepine; resulted in significant changes in the consumed amount of the standard diet, which was open available for all mice. Therefore, the obtained parameters reflect the impact of the ice cream type on the total diet eaten. Where the feeding on the curcumin ice cream increased the food intake than when the β-carotene ice cream was introduced, possibly due to the relative likeness of mice. Likewise, the lactoferrin ice cream samples were characterized with the lowest food intake values and increased the satiety of mice. While the WPI ice cream increased the appetite of mice. The treatment with tramadol led to record the highest significant values of food intake. Body weight gain (BWG) and food efficiency ratio (FER) values took similar trends to each other's and did not follow the food intake trend; proving the physiological functionality of the applied ingredients. The control group that fed on standard soy protein diet and the tramadol treated group took the lowest FER values versus the other groups which fed on the functional ice cream. These observations were in accordance with the findings of Kitka et al., [23] who reported that tramadol decreased the body weight during the diet- induced obesity test. Curcumin ice cream diet led to decrease the BWG and FER, furthermore amongst the functional proteins, lactoferrin ice cream caused the highest values of BWG and FER. Similar observation was reported by Zanzer et al., [24] who found that curcuminoids reduced early postprandial glucose levels.

Table 2: Ls means values for mice's Groups treatments on food intake, body weight gain and food efficiency ratio of mice at the end of 42-days feeding period on dried functional ice cream.

Source of variation	Food intake (g)	Body weight gain (g)	FER
	Mice's Group s		
Group 1 (Control)	$120.65^{c} \pm 2.81$	$3.36^{g} \pm 0.33$	0.0278^{g}
Group 2	$126.15^{a} \pm 1.88$	$4.85^{f} \pm 0.47$	0.0385^{f}
Group 3	$123.72^{b} \pm 1.88$	$6.84^{e} \pm 0.58$	0.0553^{e}
Group 4	$121.06^{c} \pm 1.83$	$7.55^{d} \pm 0.41$	0.0624^{d}
Group 5	$123.04^{b} \pm 0.99$	$6.34^{e} \pm 0.13$	0.0515^{e}
Group 6	$118.07^{d} \pm 1.35$	$9.15^{b} \pm 0.42$	0.0775^{b}
Group 7	$114.25^{e} \pm 1.42$	$10.34^{a} \pm 0.48$	0.0905^{a}
Group 8	$116.17^{de} \pm 1.51$	$8.14^{c} \pm 0.61$	0.0701^{c}
P-value	<0.0001	<0.0001	<0.0001

*Values were expressed as average ± standard deviation.

Behavioral evaluation of the functional ice cream antidepressant-like activity using mice's tail suspension and forced swim tests

Results of Table 3 elucidated that, the control group reflected the highest immobility time in the tail suspension test compared to all mice groups. The administration of tramadol at a rate of 40 mg/kg/day for 5 days led to attain the lowest values of the immobility time which is an indicator of the anti-despair and depression activity, followed by the group of curcumin and α-casozepine then the group of curcumin and lactoferrin. That could be ascribed to the strengthen role of curcumin in increasing the hippocampal neurogenesis. This observation was supported by the study of Xu et al., [28] who concluded that curcumin modulates the serotonin receptors and the hippocampus derived

Table 3: Ls means values for mice's groups on immobility, swimming and climbing times in tail suspension and forced swimming tests during a 42-days feeding period on dried functional ice cream.

Source of variation	Immoboility time (s)	Swimming time (s)	Climbing time (s)
Mice's Group s			
Group 1 (control)	$125^{a} \pm 1.78$*	$35^{g} \pm 1.41$	$40^{g} \pm 0.68$
Group 2	$58^{h} \pm 0.89$	$139^{a} \pm 1.09$	$34^{h} \pm 0.70$
Group 3	$84^{d} \pm 0.89$	$62^{f} \pm 0.98$	$92^{b} \pm 1.67$
Group 4	$75^{f} \pm 1.41$	$95^{b} \pm 0.63$	$74^{c} \pm 1.03$
Group 5	$69^{g} \pm 0.89$	$79^{d} \pm 1.26$	$53^{e} \pm 0.61$
Group 6	$96^{b} \pm 0.63$	$64^{e} \pm 0.89$	$100^{a} \pm 0.42$
Group 7	$88^{c} \pm 0.89$	$96^{b} \pm 0.63$	$70^{d} \pm 0.66$
Group 8	$81^{e} \pm 0.63$	$81^{c} \pm 0.89$	$49^{f} \pm 1.46$
P-value	<.0001	<.0001	<.0001

*Values were expressed as average ± standard deviation.

neurotrophic factor. The application of α-casozepine with β-carotene succeeded to reduce the immobility time in comparison with the control diet but with an efficacy lower than when the α-casozepine was administered with curcumin. That could be due to the actin of the active peptide α-casozepine in modulating the benzodiazepine site of the GABAA receptors which in turn enhanced the protein levels of GABAA receptors in hypothalamic neurons [25]. Regarding the swimming time, all the test groups reflected significant increments in the swimming time as compared to the control one. Among the test groups, the feeding on lactoferrin either with curcumin or β-carotene led to attain the highest swimming times after the tramadol group; reflecting a relatively high degree of depression resistance. That is in agreement with the findings of Takeuchi et al., [26] who reported that lactoferrin helped in increasing the plasma corticosterone in maternal separated rats. With respect to climbing time, tramadol group obtained the lowest time of all groups, followed by the control group. Feeding on the functional ice cream led to increase the climbing time and the ambition for survival. Amongst the test groups other than the tramadol and control ones, the group of WPI and β-carotene obtained the highest climbing time, followed by that of WPI and curcumin. The groups fed on α-casozepine showed relative decrements in the values of climbing time as compared to the other groups which fed on the other functional proteinous ingredients, possibly due to the strong anxiolytic effect of α-casozepine [27].

Table 4 Lsmeans values for mice's groups treatments on some neurotransmitters content in the hypothalamus brain region at the end of 42-days feeding period on dried functional ice cream.

Source of variation	Dopamine (ng/g)	Serotonin (ng/g)	Epinephrine (ng/g)	γ-aminobutyric acid (μg/g)	Glutamate (μg/g)
Mice's groups					
Group1(control)	$112.79^{bc}\pm22.5$	$283.5^{f}\pm18.56$	$187.33^{f}\pm9.64$	$400.83^{ab}\pm16.09$	$812.16^{f}\pm102.24$
Group2	$243.00^{a}\pm18.90$	$616.83^{a}\pm18.15$	$372.11^{a}\pm9.70$	$119.66^{f}\pm11.29$	$2325.50^{a}\pm111.99$
Group3	$111.50^{bc}\pm10.90$	$356.00^{bc}\pm12.80$	$304.50^{c}\pm8.06$	$387.83^{bc}\pm18.38$	$1831.00^{d}\pm109.44$
Group4	$106.39^{bc}\pm16.11$	$340.33^{cd}\pm11.94$	$283.83^{d}\pm14.21$	$403.33^{a}\pm11.91$	$1715.83^{de}\pm68.87$
Group5	$120.16^{b}\pm17.31$	$366.33^{b}\pm10.98$	$330.66^{b}\pm13.50$	$130.16^{ef}\pm7.88$	$2072.83^{b}\pm102.84$
Group6	$100.50^{bc}\pm11.64$	$334.16^{d}\pm5.87$	$285.16^{d}\pm11.99$	$368.83^{d}\pm8.75$	$1732.50^{d}\pm59.11$
Group7	$97.66^{c}\pm14.29$	$314.00^{e}\pm17.11$	$256.00^{e}\pm9.87$	$376.16^{cd}\pm6.43$	$1604.83^{e}\pm117.63$
Group8	$108.16^{bc}\pm9.40$	$341.00^{cd}\pm7.40$	$307.33^{c}\pm22.58$	$142.16^{e}\pm7.96$	$1958.66^{c}\pm82.96$
P value	<.0001	<.0001	<.0001	<.0001	<.0001

*Values were expressed as average ± standard deviation.

Biochemical evaluation of the functional ice cream antidepressant-like activity

Data shown on Table 4 elucidated that, no clear trends could be established in the hypothalamus dopamine content between the mice groups, as the tramadol group obtained the highest significant value, and the lowest value was for the group of lactoferrin and β-carotene, while the other groups were statistically similar to the control group. That could be ascribed to the relatively low content of tyrosine and phenylalanine in lactoferrin. With regard to the serotonin content, all the test groups showed significant increments in comparison to the control. Moreover, the group of curcumin and α-casozepine was distinguished by the highest serotonine level of the test groups after the tramadol group. It's noteworthy to mention that the functional ice cream helped in raising and normalizing the levels of serotonine gradually and slightly, unlike tramadol which caused a rapid and relatively high raise in the hypothalamus serotonine level. This rapid raise may cause adverse effects like affecting the physical activity of mice as confirmed by the results of the forced swimming test. Concerning the epinephrine contents, the results revealed that the feeding on curcumin or β-carotene together with the milk derived functional proteins led to attain a slight significant increments in the hypothalamus epinephrine of the experimental mice. The highest increase after the tramadol group was for the group of curcumin and α- casozepine, whereas the lowest increase was for the group of β-carotene and lactoferrin. The results of γ-aminobutyric acid indicated that the tramadol group gained the lowest value. On the other hand, the groups fed on β-carotene ice cream samples obtained lower γ-aminobutyric acid levels versus the other groups fed on curcumin containing ice cream. These phenomena are supported by the findings of Amengua et al., [28]. Amongst the functional proteins, the administration of α-casozepine led to attain the lowest values of γ-aminobutyric acid. The hypothalamic glutamate levels were increased in all experimental groups upon either feeding on the functional ice cream or application of tramadol; this increase in the release of glutamate could be considered as a driving force for the antidepressant-like effect as previously concluded by Machado-Vieira et al., [29]. Among the applied functional protein ingredients, α-casozepine caused the highest values of glutamate especially when mixed with curcumin.

CONCLUSION

The study findings led to conclude that feeding mice on the dried functional ice cream containing the studied milk derived active proteinous substances and curcumin or β-carotene as natural phytochemical pigments showed strong antidepressant-like activity behaviourally and biochemically. Mixing curcumin and α-casozepine was the most distinguished feeding style reflecting the best decrease in the immobility time and the optimum gradual increase in serotonin, epinephrine and glutamate levels; unlike tramadol which caused a sever increase in the hypothalamus monoamines and led to impair the mice physical activity. All the used active materials besides their role in the antidepressant-like activity, helped to improve the physicochemical quality of the vanilla ice cream.

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