Global Veterinaria 14 (4): 478-489, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.04.93213

Polyphenon E Could Improve Negative Changes Caused by Chronic Mild Stress in Male Wistar Rats

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Abstract: The aim of the present study is to examine behavioral and hormonal changes in chronic mild stress (CMS) depression model in male Wistar rate and evaluate the possible effects of Polyphenon E (PolyE) therapy. CMS (for 40 days) was applied; Poly E (5g/"L" or 10g/"L") was administered during CMS. To evaluate behavioral changes, forced swimming test (depression) and plus maze test (anxiety) parameters was calculated. The sucrose intake test and food intake was also evaluated. At the end of the experiment plasma testosterone and relative organs weight (brain, adrenal and testes) were assessed. The CMS decreases testosterone levels, increases relative adrenal weight and generated in animals depressogenic-like, anxiogenic-like behavior and the loss of succrose and food intake. Contrary, the administration of Poly E1 (5g/"L") to CMS group seems to reverses adrenal weight and testosterone levels and causes antidepressive-like and anxiolytic-like behavior. Polyphenon E could improve negative changes caused by CMS.

Key words: CMS · Polyphenon E · Epigallocatechin-3-gallate (EGCG) · Behavior · Depression

INTRODUCTION

It has been well established that chronic stress is crucially related to the pathophysiology of mood disorders [1]. Major depression is a mood disorder characterized by clinical symptoms listed in DSM IV such as anhedonia (an incapacity to experience pleasure) [2], neurochimical changes [3] and endocrine disease [4, 5]. Two of the most important hormonal changes related to depression are a reduction in testosterone (T) levels and a stress-dependent sustained increase of plasmatic glucocorticoids, due to an impaired negative feedback in the hypothalamic–pituitary–adrenal axis (HPA) [6,7].

Among the complex interplay of multitudinous pathophysiological factors of depression, oxidative stress has been reported to play a key role. The theory of oxidative stress in depression can be explained by the concept that excessive amount of free radicals are toxic to the neuronal cells and can affect the physiological activity of the brain [8]. The accumulated reactive oxygen species are highly unstable in nature and have potential to damage cellular proteins, lipids and nucleic acids [8]. The endogenous antioxidant enzymes have free radical scavenging action [8]. However, enhanced accumulation of free radicals due to increased production and/or deficiencies of antioxidant defense results in oxidative stress in the brain that culminate in the development of psychological deficits such as depression [9].

In experimental psychiatry, animal models are an important tool to study neuropsychological theories of specific disorders, such as depression [10]. Accordingly, an animal model has been developed by Willner *et al*. [10] using Chronic mild stress (CMS) in rodents to mimic the pathophysiological changes associated with stressful events that can invoke depression-like behavior. The paradigm (CMS) consists of several mild stressors which are analogous to those associated with humans. In this paradigm, animals are subjected to a variety of mild stressors every day. After several weeks, the animals show a decrease in consumption of a palatable sweet solution, known as anhedonia, which is the core character of depression [10].

Antioxidants are molecules which can interact with free radicals and break the chain reaction before vital molecules are damaged. It has been suggested that fruits and vegetables may play an important role in delaying the onset of some illnesses, such as certain cancers, cardiovascular diseases and degenerative diseases and

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about the possible therapeutic value of antioxidants against these illnesses [11]. To date, over 3000 flavonoids have been identified. These can mainly be found in the pigments in flowers or in leaves [12]. Flavonoids are primarily known for their anti-oxidative, vasculoprotective [13], anti-inflammatory and antidiabetic properties. Green tea contains a considerable amount of polyphenolic compounds and green tea polyphenols have aroused increasing attentions in recent years for protecting brain's functions. Green tea contains multiple catechin components, though epigallocatechin-3-gallate is the primary catechin accounting for 50–80% in a brewed cup [14]. Epicatechin-3-gallate is the second most concentrated catechin component of green tea and is associated with its antiinflammatory/antioxidant properties [11]. Other major catechins found in green tea include epicatechin and epigallocatechin. Polyphenon E (PolyE) is a well defined pharmaceutical grade mixture of polyphenols that contain at least five different catechins: epicatechin, gallocatechin gallate, epigallocatechin, epicatechin gallate and epigallocatechin-3-gallate (EGCG), with EGCG being the most abundant [15].

The aim of the present study is to examine behavioral and hormonal changes in CMS depression model in Wistar male rate and evaluate the possible effects of Polyphenon therapy.

MATERIALS AND METHODS

Experimental Animals: Adult male Wistar rats (purchased from Pasteur Institute, Algiers) weighing between 200 and 300 g were used. The rats were housed in polypropylene cages with wood shavings as bedding, under controlled room conditions of light (12 "h" light–dark cycle, lights on at 07:00 am) and temperature (22±2°C), with free access to food and water. Animals were softly handled 3 to 5 minutes per day by the experimenter before the onset of the experiment.

After an acclimatization period of 15 days, 32 male rats were divided into 4 groups: a control group non stressed animals (n=8), a group of 8 rats submitted to chronic mild stress (CMS) for 6 weeks, a group of 8 rats chronically stressed and treated with Poly E1 (5g/"L") (CMS+PolyE1) and group of CMS+PolyE2 (10g/"L") (n=8). Poly.E was administered for 40 days at a constant volume 5g/"L" (Poly E1) and 10g/"L" (Poly E2) except prior to the sucrose preference test or when they were submitted to chronic mild stress. The experimental protocol of CMS was shown in Table 1.

Table 1: Program of stressors and duration applied every day

Days	Stress factors	Duration
1	Water deprivation	24 h
2	Food deprivation	24 h
3,4,5	Isolation	24 h
6	Flashing light	3 h
7	Food deprivation	24 h
8	Forced swimming	10 min
9	Wet litter	1 h
10	Water deprivation	24 h
11, 12	No stress	-
13	Wet litter 4°C	2 h
14	Flashing light	2 h 1/2
15	Food deprivation	24 h
16	Forced swimming	15 min
17, 18, 19	Isolation	24 h
20	Water deprivation	24 h
21	Food deprivation	24 h
22	Flashing light	3 h
23	Wet litter	1 h
24, 25	Isolation	24 h
26	Wet litter 4°C	1 h 1/2
27	Forced swimming	10 min
28	Flashing light	3 h 1/2
29	No stress	-
30	Food deprivation	24 h
31	Wet litter	1 h
32	Flashing light	2 h
33	Water deprivation	24 h
34	Wet litter 4°C	2 h
35	Forced swimming	10 min
36, 37	Isolation	24 h
38	Water deprivation	-
39	Flashing light	3 h
40	Forced swimming	10 min

The depression-related behaviours were assessed before (baseline) and after the CMS procedure by the elevated plus-maze (EPM) and Forced swimming test (FST). The experimental protocol was approved by the Scientific Committee of our faculty which is consistent with the principles of Animal Health (NIH Publication No. 85-23, revised 1985).

Food Intake: The standard food in the form diets especially for rats (Oravi el Harrouch, Skikda Algeria) is supplied to the animals. The food intake was performed in the rat's home cage. Pre-weighted bottle containing food was presented to each rat. The bottle was weighted again after 8h and the weight difference was calculated. The food intake was realized in days 3, 8, 16, 22 and 31.

Sucrose Preference Test: All the rats were submitted to 48 h of forced exposure to 1% sucrose solution in order to habituate to it, during which sucrose solution was the only fluid available for consumption, followed by two days of free access to food and water. After this, the rats were submitted to water deprivation for 16 "h" prior to performing the sucrose preference test; baseline test at day zero. The sucrose preference test was realized in days 0, 11, 21, 34 and 39. The sucrose preference test [16] was performed in the rat's home cage: two pre-weighted bottles, one containing tap water and another containing 1% sucrose solution, were presented to each rat. The bottles were weighed again after 1 "h" and the weight difference was considered to be the rat intake from each bottle. The sum of water and sucrose intake was defined as total intake and the sucrose preference was expressed as the percentage of sucrose intake from the total intake following the formula: % sucrose preference= sucrose intake X100/total intake.

Chronic Mild Stress: Chronic mild stress (CMS) was used according to the literature [17]. The 3 groups CMS, CMS+PolyE1, CMS+PolyE2 were submitted to 40 days of chronic mild stress. Several stressors have been used: food deprivation, water deprivation, forced swimming, flashing light, isolation, wet litter and wet litter at 4°C. The stress was applied at times that change every day, in order to minimize its predictability. Control rats were kept intact in their cages for 40 days, receiving only usual care, food and water.

Polyphenon E Administration: PolyE is a well defined pharmaceutical grade mixture of polyphenols that contain at least five different catechins: epicatechin, gallocatechin gallate, epigallocatechin, epicatechin gallate and epigallocatechin-3-gallate (EGCG), with EGCG being the most abundant [15]. In this study, stressed animals were exposed to both PolyE1 (5g/"L" in water) and Poly E2 (10 g/"L"). Polyphenon E was obtained from Tokyo Food Techno (Tokyo, Japan).

Body Weight Gain: Body weights were measured weekly during the CMS procedure. The body weight gain was calculated as the difference between the final and baseline (day 0) body weight.

Behavioral Study

Elevated plus Maze Test: The elevated plus maze test [18] is used to measure the degree of anxiety in rodents. It is composed of four arms (50 × 10cm), two open arms perpendicular to two closed arms with 40cm high walls of Plexiglas. The intersection of the four arms (central area) is a square of 10 × 10cm. The apparatus was elevated of 50cm from the ground. The test was performed for 5 min by placing the animal in the central area facing an open arm. Since the rate fears the empty and high spaces, his exploration of open arms shows a less anxious behavior. On the contrary, the more the animal remains in the closed arms, his behavior are known to be anxious. The 5 min sequences were recorded by a video camera to measure the following parameters: Open arms duration, closed arms duration, Open arms entries, closed arms entries and Freezing duration.

Forced Swimming Test: The forced swim test [19] is a behavioral test of inducing despair in rats by placing the animal 15min in a glass aquarium 54 cm height (34 × 60cm). This dimension ensures that the rat can´t escape by climbing to the edges of the device. The aquarium is filled with water (26 °C) to a height of 40 cm, in order to ensure that the rat will not use his legs to keep the surface and thus force him to swim. The procedure of forced swimming (FST) in rats occurs in two phases: the pre-test and test, separated by an interval of 24 hours. During the pre-test, the rat was placed for 15 min. At the end of session, the animal is immobile. The next day, the animal plunged into the aquarium for 5 min. The swimming session on each day was videotaped for behavioral analysis. The time of immobility, swimming and climbing are calculated.

Blood Sample and Organs Collection: Retro-orbital blood samples were collected. Blood samples were centrifuged at 5000 r/min to be used for hormone assays of testosterone, which measured these levels by the BIOTECH conventional ELISA test kit, with a TECAN ELISA reader equipped with Magellan computer software that automatically calculates the standard range and provides the value of the hormone to the desired unit directly. The rats were killed under slight ether anesthesia at the end of the experiment. The testes, adrenal glands and brain were removed, cleared of adhering connective tissue and weighed.

Statistical Analysis of Results: Data are presented as mean ± SEM. Data were analyzed by one-way ANOVA or two-way ANOVA and Newman and Keuls or Benferroni as the post hoc test. Results were considered significant at $p < 0.05$. Graph Pad Prism 5 for windows version 5.01 was used to do the analysis.

RESULTS

Effect of CMS and CMS+Poly E on the Depressive-Like Behavior: One-way ANOVA was condcuted to compare the effect of CMS and CMS+ Poly E on immobility, climbing and swimming times in the FST. Benferroni multiple comparison test was used to compare groups to another.

There was a significant effect on immobility time Benferroni test indicates that CMS induced a depressive-like behavior evident as a significant increase in immobility time in the FST (Control vs CMS: t= 3.404, $p<0.05$). Treatment of stressed rats by poly E produces antidepressant-like behavior effect explained by the reduction of immobility time (Control vs CMS+Poly E1: t= 9.844, $p<0.0001$); (Control vs CMS+Poly E2: t= 5.674, $p<0.0001$); (CMS vs CMS+Poly E1: t= 13.25, $p<0.0001$); (CMS vs CMS+Poly E2: t= 9.078, $p<0.0001$).

We also see that there was a significant effect on climbing time (R squared = 0.8958, F=80.23, $p<0.0001$). Benferroni test indicates that CMS induced a significant decrease in climbing time in stressed animals compared to Control group (Control vs CMS: t=3.826, $p<0.001$). Climbing time increases in CMS+Poly E1 group compared to Control and CMS groups (Control vs CMS+Poly E1: t=7.835, $p<0.0001$); (Control vs CMS+Poly E2: t= 9.475, $p<0.0001$); (CMS vs CMS+Poly E1: t= 11.66, $p<0.0001$); (CMS vs CMS+Poly E2: t= 13.30, $p<0.0001$). Finally, there was a significant effect on swimming time (R squared = 0.8207, F=42.73, $p<0.001$). Benferroni test indicates that CMS+Poly E induced a significant increase in swimming time compared to stressed animals (CMS vs CMS+Poly E1: t= 10.55, $p<0.0001$); (CMS vs CMS+Poly E2: t= 4.134, $p<0.001$).

Anxiety-Like Behavioral Effects of CMS and CMS+Poly E in the Elevated Plus-Maze Test: One-way ANOVA was used to compare different groups. Benferroni multiple comparison test was used to compare groups to another.

The analyse of time spent in open arms revealed a significant effect of treatments (R squared = 0.9276, F=119.7, $p<0.0001$). Treatment (CMS and Poly E) also has an effect on the number of entries in the open arms (R2= 0.8362, F=47.65, $p<0.0001$) and the immobility time (R2 = 0.9413, F=149.7, $p<0.0001$).

Stressed rats spent less time in the closed arms and there come less often. This demonstrates that the CMS leads to an increase of anxiety in animals. CMS decreased motor activity as evidenced by significantly higher time of immobility. Conversely Poly E, regardless of the dose, resulting in an increase of locomotion.

Temporal Evolution of the Weight Changes (Gram) Measured in CMS and CMS+Poly E: Two-way ANOVA test indicates that the treatment has no effect (F= 1.752, $p = 0.1995$), by against, the days factor has an effect (F= 18.17, $p < 0.0001$). CMS induced a marked body weight at the end of the experiment (day 50). This decrease is not statistically significant.

Temporal Evolution of Food Intake (G/100g of Body Weight) in CMS and Poly E groups: Two-way ANOVA indicated a significant difference on the food intake (Control vs CMS) in days (D03, D08) ($p<0.001$) (D22, D31) ($p<0.001$) but no significance difference D16. (Control vs CMS+Poly E1) we notice significant difference in days (D0, D03, D08, D16) ($p<0.001$) and in the D22 we have significance difference ($p < 005$). In the last day D31 no significance difference. About the group (Control vs CMS+Poly E2) we note a significance difference in days (D0, J03, D08) ($p<0.001$), D16 no significance difference, days (D22, D31) significance difference (P<0.001). the group (CMS vs CMS+Poly E1) presents a significance difference in days (D0, D03, D08, D16, D22) ($p<0.001$), (CMS vs CMS+Poly E2) days (D0, D03, D08) a significance difference ($p<0.001$), days (D16, D22) no significance difference ($p > 0.05$), D31 a significance difference ($p<0.001$). Finally the lasts groups (CMS+Poly E1 vs CMS+Poly E2) a significance difference ($p<0.001$) all days of treatment (D0, D03, D08, D16, D22, D31).

Temporal Evolution of Sucrose Intake (%) in CMS and Poly E groups: Two-way ANOVA indicated a significant effect of treatment (F=342.1, $p<0.0001$), Days (F=353.3, $p<0.0001$) and interaction (F=177.8, $p<0.0001$). Benferroni post-tests was used to compare each column (treatments) to all the others columns. The test revealed a significant differences between Control vs (CMS, CMS+Poly E1 and CMS+Poly E2) on day 00, day 21 and day 34. No significant seen between Control and CMS+Poly E1 on day 39.

Effects of CMS and CMS+Poly E in Testosterone Levels: CMS significantly decreased relative testosterone levels (F=21.95, R squared=0.7016, CMS vs control: q=10.32, $p < 0.0001$). This decrease is reversed by Poly E1 treatment (CMS+Poly E1 vs control: q=2.846, NS).

Effects of CMS and CMS+Poly E in Organ Weights: According to Newman-Keuls multiplie comparaison Test method there is significant differerence in group (Control vs CMS) ($p < 005$) but (Control vs CMS+Poly E2) no significant difference is found regarding the (CMS + Poly E2 vs CMS) we notice a significant difference ($p < 005$).

Table 2: Bonferroni's Multiple Comparison Test. Evolution of the weight changes (gram) measured in CMS and CMS+Poly E. (*p <0.05; ** p <0.01; ***p<0.001)

Days	D0	D10	D20	D30	D40	D50
Control vs CMS (t, p value)	0	1.19	1.432	2.42	0.963	0.765
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns
Control vs CMS+Poly E1 (t, p value)	0	0.07409	1.432	1.408	1.467	0.829
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns
Control vs CMS+Poly E2 (t, P value)	0	0.5927	0.07409	1.605	0.321	2.233
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns
CMS vs CMS+Poly E1 (t, P value)	0	1.264	0	1.013	0.5038	0.064
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns
CMS vs CMS+Poly E2 (t, P value)	0	0.5976	1.506	0.815	0.6421	1.467
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns
CMS+Poly E1 vs CMS+Poly E2 (t, P value)	0	0.6668	1.506	0.1976	1.146	1.403
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	ns	Ns	ns	ns	ns	ns

Table 3: Bonferroni's Multiple Comparison Test. Sucrose intake (%) in CMS and Poly E groups (*p <0.05; ** p <0.01; *** p <0.001)

Days	00	11	21	34	39
Control vs CMS (t, p)	3.036	1.14	22.58	11.17	23.47
	*	ns	***	***	***
Control vs CMS+Poly E1 (t, p)	4.416	1.14	25.72	30.82	1.74
	***	ns	***	***	ns
Control vs CMS+ Poly E2 (t, p)	3.195	1.486	0.052	5.457	19.65
	**	ns	ns	***	***
CMS vs CMS+Poly E1 (t, p)	1.38	0	3.135	19.66	21.73
	Ns	ns	*	***	***
CMS vs CMS+ Poly E2 (t, p)	0.1588	0.345	22.64	5.708	3.812
	Ns	ns	***	***	**
CMS+Poly E1 vs CMS+ Poly E2 (t, p)	1.221	0.345	25.77	25.37	17.91
	Ns	ns	***	***	***

Finally (CMS+Poly E2 vs CMS +Poly E1) and (CMS + Poly E1 vs CMS) we have not significant difference. (Control vs CMS) significant difference (p < 005), (Control vs CMS+Poly E1, Control vs CMS+Poly E2) no significant difference, (CMS+Poly E2 vs CMS) significant difference (p < 005), (CMS+Poly E2 vs CMS+Poly E1, CMS+Poly E1 vs CMS).

DISCUSSION

The CMS model has been extensively used by many researchers, their results have shown that rodents exposed to chronic stress reduce their sucrose intake after some weeks [17]. Our results confirm this observation when chronic stress for 40 days generated in animals depressogenic-like and anxiogenic-like behavior effects manifested by the reduction of immobility time (in the forced swimming test), decreasing of locomotion, time spent and number of entries in the open arms (in the plus maze test) and the loss of succrose intake (in preference succrose test) called anhedonia behavior. These changes indicated depression-like changes in CMS rats and demonstrated the feasibility of the depression model.

These behavioral findings are corroborated with other weight and hormonal parameters, in this case, plasma T levels and the relative weights of the adrenals, brain and testes. The chronic mild stress caused a significant increase of adrenal weight accompagned by reduction of T rate. Contrary, the administration of Poly E1 at a dose (5g/"L") to CMS group seems to reverse adrenal weight and T levels and causes antidepressive-like and anxiolytic-like behavior.

Increasing attention is being paid in recent years to explain the origin of the decline of T in stressed animals. The mechanism by which is affected has not yet been

clarified. Stress is associated with detrimental effects on male reproductive function. It is known that stress increases reactive oxygen species generation in the male reproductive tract [20]. In this study, it was shown that chronic cold stress causes a decrease in testicular absolute weight, associated increasing of reactive oxygen species and lipid peroxidation production at 20, 40 and 50 days. The activity of superoxide dismutase and glutathione peroxidase decreased throughout the duration of chronic stress and the activity of catalase decreased at 40 and 50 days and increased at 20 days. The expression of copper/zinc superoxide dismutase and catalase were not modified, but the expression of phospholipid hydroperoxide glutathione peroxidase decreased at 20 days. These results suggest that during acute chronic cold stress equilibrium redox is lost, with low enzyme activity but without modifying their expression. In addition, corticosterone increased while T decreased [20].

Psychological stress activated HPA which resulted in the increasing release of glucocordicoids. Many studies inferred that endogenic glucocorticoids played an important role in the pathological impairments induced by stress [21]. For a long time, it was recognized that the T reduction could be associated with a dysfunction of the adrenal HPA axis. Indeed, the gonadotropic axis is inhibited by all the various levels components of the HPA axis [7,22, 23]. The hypothalamus, CRH (corticotropin releasing hormone) inhibits GnRH (gonadotropin releasing hormone) hypothalamic in arcuate nucleus. This effect could be also mediated by β-endorphin from the split of proopiomelanocortin (POMC) under the effect of hypothalamic CRH [24]. In addition, glucocorticoids exert inhibitory effects on hypothalamic GnRH neuron and the gonads in causing decreased sensitivity of target tissues to sex steroids (Peripheral resistance) [22].

In this study, we examined the neuropharmacological effects of Poly E in the Forced swimming test (FST). There FST is an animal model of depression, is a aversive stressful situation where rats cannot escape and produces immobility [19]. During the FST, rats show active behavior (swimming and climbing), as well as passive (immobility). The molecules which decrease the duration of immobility in the FST are considered as effective antidepressants [19]. In our study, we found that Poly E1 (5g/"L") decreases immobility time. This indicates an antidepressant-like effect of these drugs. However, this decrease in immobility time can be either in favor of an increase in swimming time. Theoretically this explains why Poly E1 exerts its anti depressive-like effect by acting on the serotoninergic route [7,25]. The plus Maze test is one of the more behavior models popular for anxiety. Increasing the number of entries and the time spent in the open arms are considered the most indexes representative of anxiolytic-like activity. In this device, the rats prefer normally spend much of their time in the closed arms. This behavior seems to reflect an aversion to the open arms which is generated by the fear of open spaces. Drugs that increasing exploration of open arms is considered anxiolytics and the reverse is true for anxiety [26]. At the maze test, we found that the Poly E1 exercises anxiolytic-like effect.

These behavioral effects can have several origins. Either the Poly E1 could act directly by changing the oxidative status, or that its effects are mediated by corticosterone and/or T. As such, severed studies showned that T plays an important role in brain dysfunction associated with stress. Firstly, T levels are positively correlated with cognitive impairment [27]. Moreover, it appears that T exerted beneficial effects in brain function, including preventing neuronal cell death, balancing brain oxidative stress and antioxidant activity, improving synaptic plasticity and involving cognitive formation [27]. Due to its lipophilic nature, T readily crosses the blood brain barrier and acts directly in the brain by genomic pathway. Indeed in a very recent study by Filová *et al*. [28], it was shown, among gonadectomized rats that T has a non rapid effect compared to estradiol which appears to have a rapid effect mediated by a non-genomic pathway. In another study, it was reported that both T and estradiol exhibit anxiolytic- and antidepressant-like effects in gonadectomized male rats, while similarly regulating critical mediators of these behaviors, suggesting common underlying mechanisms [29]. In this study, it is suggested that T's protective effects are mediated, in part, by its aromatization in the dentate gyrus [29].

It's has been shown that during the forced swimming, corticosteroid hormones regulate the immobility time [30, 31] noted an increase in corticosterone accompanied by an increase of immobility time in forced swimming test. Surgical adrenalectomy results in decreased immobility time [30, 32, 33]. However, the administration of corticosterone (1 mg /kg) the first day of test after 15 minutes (first FST), increases the second immobility time test day (2nd FST). Dexamethasone (dose-dependent) abolished this effect [30, 33]. The metyrapone (120 mg /Kg) (blocks 11beta-hydroxylase) injected 3 hours before the first FST reduced significantly the immobility time. This effect is donated by the corticosterone (dose-dependent).

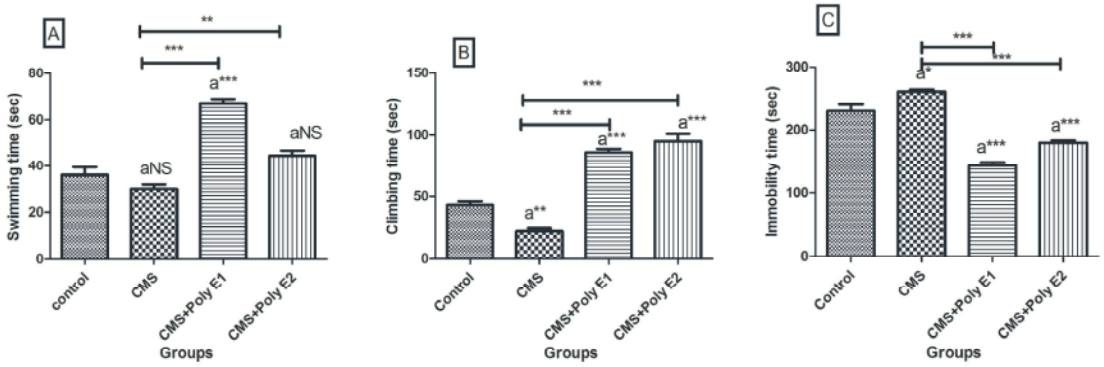

Fig. 1: Effect of CMS and CMS+Poly E on the depressive-like behavior (A: Swimming time, B: Climbing time, C: Immobility time). One-way ANOVA and Benferroni multiple comparison test post hoc test were used to compare groups to another (*p<0.05, **p<0.01, ***p<0.001)

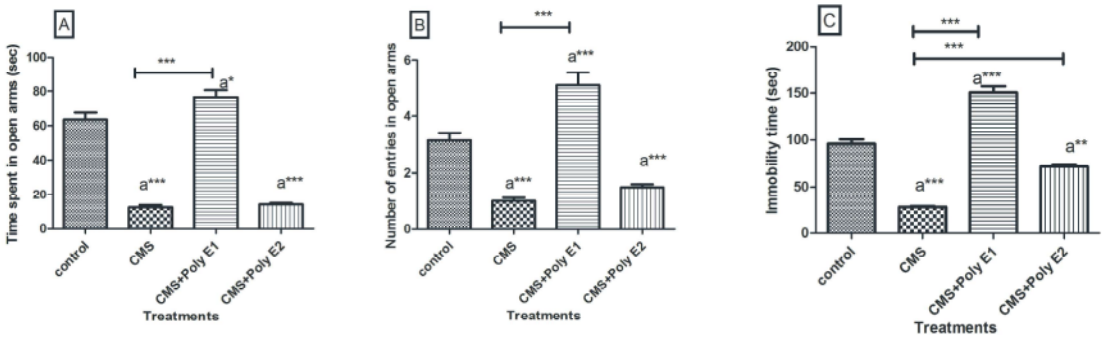

Fig. 2: Anxiety-like behavioral effects of CMS and CMS+Poly E in the elevated plus-maze test (A: Time spent in open arms ("s"), B: Number of entries in open arms, C: Immobility time ("s"). One-way ANOVA was used to compare different groups followed with Benferroni multiple comparison test to compare groups to another (*p<0.05, **p<0.01, ***p<0.001)

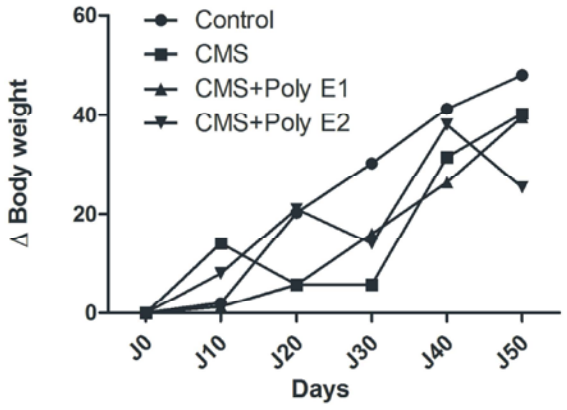

Fig. 3: Temporal evolution of the weight changes (gram) measured in CMS and CMS+Poly E. Two-way ANOVA test treatment X Days and Benferroni post-tests were used to compare each column (treatments) to all the others columns (*p<0.05, **p<0.01, ***p<0.001)

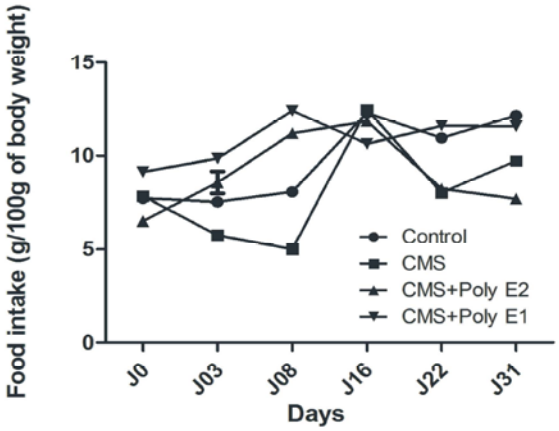

Fig. 4: Effects of CMS and Poly E on the food intake (g/100g of body weight) measured in CMS and CMS+Poly E. Two-way ANOVA test treatment X Days and Benferroni post-tests were used to compare each column (treatments) to all the others columns (*p<0.05, **p<0.01, ***p<0.001)

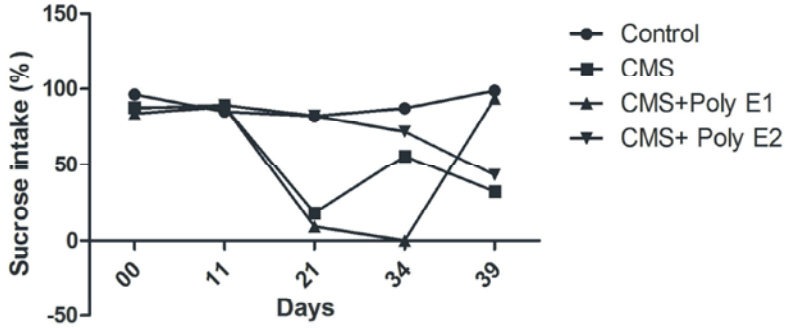

Fig. 5: Temporal evolution of sucrose intake (%) in CMS and Poly E groups. Two-way ANOVA treatment X Days. Benferroni post-tests was used to compare each column (treatments) to all the others columns. (*p<0.05, **p<0.01, ***p<0.001)

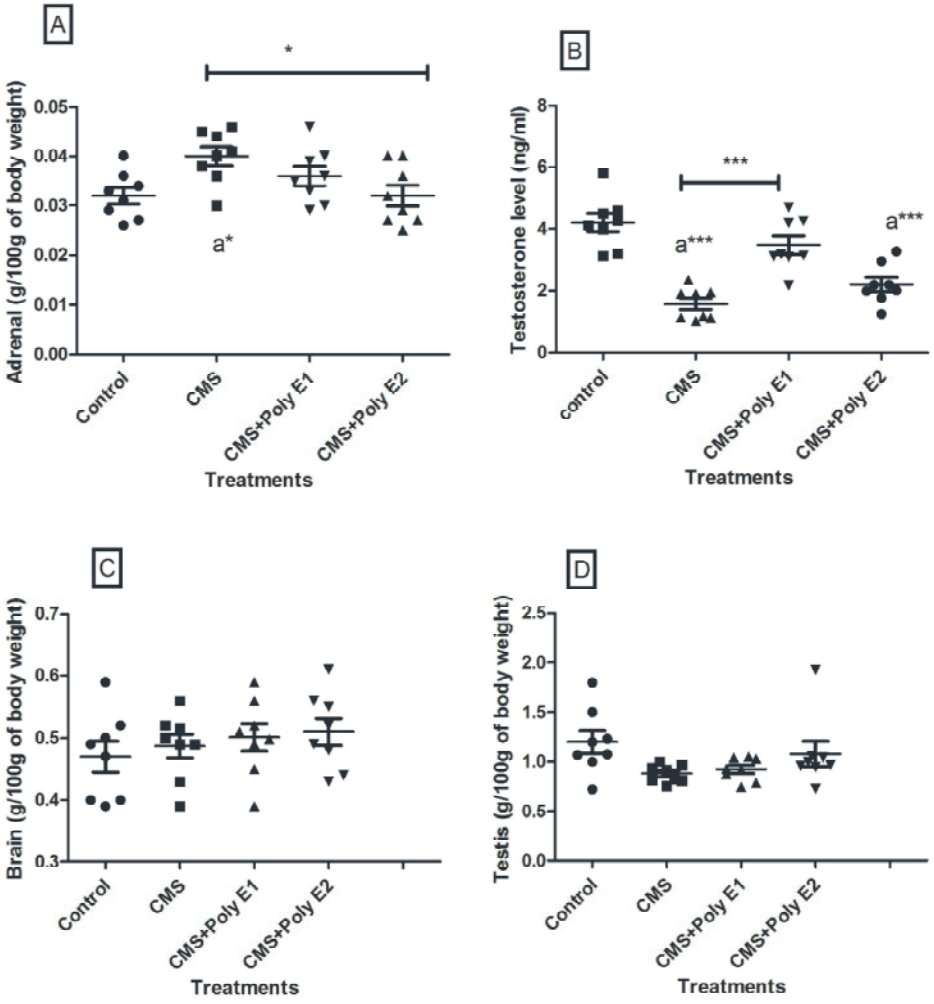

Fig. 6: Effects of CMS and CMS+Poly E in organs weights (g/100g of body weight)(A: Adrenal, C: Brain, D: Testes) and testosterone level (ng/ml)(B). One-way ANOVA and Newman-Keuls multiplie comparaison post hoc test were used (*p<0.05, **p<0.01, ***p<0.001).

There is experimental evidence showing that gonadal hormones steroids T and estradiol have neuroprotective effects [34]. For example, these steroids inhibit stress-induced neurodegeneration, primarily in the hippocampus [35, 36], a brain structure involved in the etiology of depression [37]. It has been suggested that depression is associated with the reduction in hippocampal cell neurogenesis [36]; thus, the higher capacity of stressed rats to develop anhedonia could be explained by plastic changes in the brain. Indeed, recent research indicate that resilience to CMS is associated to the expression of alpha and beta SNAP proteins in the hippocampus and to the expression of genes involved in hippocampal signaling [39], suggesting that the development of anhedonia after exposure to CMS is related to an impairment of hippocampal neurogenesis or cellular plasticity pathways [38, 39].

In old animals, the brain alterations that lead to a higher capacity to develop experimental depression could be underlain by age-related low levels of T and eostradiol; which impair biological processes related to neuroplasticity and neuroprotection [40]. In this line, some studies have shown an age-dependent decreased production of neurotrophic factors such as BDNF [41], which in turn is modulated by eostradiol [42] and an age-dependent reduction in hippocampal neurogenesis, effectively restored by adrenalectomy. Furthermore, it has been observed that eostradiol stimulates hippocampal neurogenesis [44]. In line with this idea, there are many clinical studies showing that low T levels are associated to an increased capacity to develop depression [45,46]. Although most of these studies consider hypogonadal patients, the results support the idea that there is an inverse relationship between T levels and depression. As we have pointed out above, that polyphenon E (PolyE) contains at least five different catechins: epicatechin, gallocatechin gallate, epigallocatechin, epicatechin gallate and epigallocatechin-3-gallate (EGCG), with EGCG being the most abundant [15]. EGCG acts as an antioxidant in the biological system [47] and attenuates lipid peroxidation caused by various forms of free radicals [48]. In particular, EGCG reduces neuronal cell death caused by transient global ischemia [49], Aβ-induced neurotoxicity and α-amino-3-hydroxy-5-methyl-4-isoxazolo propionate-induced calcium influx and neuronal cell damage [50], all of which are associated with increased oxidative stress. It was reported that long-term administration of green tea catechins reduces hippocampal lipid peroxidation and reactive oxygen species levels and increases the ferric-reducing antioxidation power of plasma in rats. These changes demonstrated improved age-related cognitive decline in rats [51].

Metabolism of green tea catechins has been studied in animal [52] and human [53, 54] subjects. After oral administration, EGCG is detected as free EGCG, its conjugates or both and peaks at 1–2 "h" after dose administration in rat systemic circulation [54,55]. Studies with radioactively labeled EGCG in mice [56] or chemiluminescence-based detection in rats also demonstrated its incorporation into the brain and into other organs, such as the kidney, heart, liver, spleen and pancreas. EGCG has a stronger antioxidant activity as compared with either vitamin E or C on a molar basis *in vitro* [11]. Furthermore, in reducing ferrous ion-induced lipid peroxidation, the IC50 values of several antioxidants are as follows: 3.32 μM for EGCG, 75.65 μM for trolox, 7.63 μM for lipoic acids and 15.48 μM for melatonin [54, 57].

It was reported by Abdul *el al*. [58] that the intake volume of Poly E-mixed water was approximately 60 ml/kg/day in the 0.5% Poly E1 group. Based on this water volume intake, a person (with a body weight of 50 kg) would have to drink about 3 "L" of Poly E per day to get similar effects. However, humans consume antioxidants (including vitamins A, B, C and E as well as polyphenols, etc.) from various food sources everyday. Therefore, a lower amount of 0.5% Poly E1-mixed water volume intake may be effective in humans to ensure the similar effects. However, detailed investigation is certainly required to understand the fate of catechins in humans.

CONCLUSION

Our results suggest that long-term administration of Poly E prevents negative behaviour changes probably caused by oxidative stress, at least by facilitating antioxidative defenses. However, further research is required to clarify the exact mechanism of how Poly E contributes to the prevention of damages in CMS depression model.

ACKNOWLEDGMENTS

This work was done as part of the national research project in Algeria (CNEPRU).

Authors' Contributions: Hacène FRIH: writing and analysis; Bachir ALI RACHEDI: writing and analysis; Samir DJEMLI: Statistical ANALYSIS and Graphics; FRAIA Asma and Zouiche Sabrina: execution of the protocol.

Conflict of Interest Disclosure: No conflict of interest

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