The Chronic Administration of Persian Herbal Formulas Psycodigest Induced The Antidepressant-Like Effect in a Chronic Unpredictable Mild Stress (CUMS) Mouse Model

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Abstract

Depression is one of the most common mental disorders. Despite the numerous medications, there are still the limitations such as side effects and treatment-resistance in depression treating. Herbal medicine has been considered as supplemental and alternative therapy for psychiatric disorders. Psycodigest is an antidepressant Persian formula composed of *Aloysia triphylla*, *Citrus aurantium*, *Echium amoneum*, *Lavandula angustifolia*, *Melissa officinalis*, *Salix aegyptiaca*, *Valeriana officinalis*, *Viola odorata*, and *Cinnamomum zeylanicum*. This survey was performed to evaluate the effect of the chronic administration of Psycodigest in chronic unpredictable mild stress (CUMS) mice behavior and its safety. CUMS-mice orally administered with Psycodigest (0.23ml /mice), Fluoxetine (20mg/kg), and Bupropion (15mg/kg) for 4 weeks. The antidepressant effects were evaluated by sucrose preference, tail suspension, and forced swimming test. The anxiolytic effect was tested by elevated plus maze. Besides, the brain, kidney, liver super oxidase dismutase (SOD) activity, and plasma level of cortisol, creatinine and alanine aminotransferase (ALT) were detected. The histopathological effects evaluated on kidney, liver, intestine and brain. The daily oral administration of Psycodigest as well as Fluoxetine and Bupropion had antidepressant, but no anxiolytic effect. Psycodigest did not change the serum level of cortisol, ALT and creatinine. Also hematological and histological examinations were not affected. Meanwhile, the brain SOD activity increased followed by Psycodigest treatment, the kidney and liver SOD did not change. The chronic administration of Psycodigest significantly ameliorated the depressive-like behaviors and showed the antioxidative effect in the brain with no toxicity in the liver and kidney.

Introduction

Depression is one of the most common mental disorder which is characterized by sadness and low mood. Depression has high recurrence, suicidal thoughts, and incidence. Over 300 million people suffer from depression in the world (WHO 2017). Despite the existence of various antidepressants, the treatment of depression is still associated with some obstacles. Side effects, inefficacy, the long latency period to the onset of action for some antidepressant drugs (Machado-Vieira et al. 2010) and treatment-resistant (Dupuy et al. 2011) make the serious need to explore the new more effective, and safe antidepressant.

Herbal medicine has been commonly recommended as a complementary and alternative therapy for psychiatric disorders. Some Medicinal plants are safer than the common antidepressants (Sarris 2018; Kolouri et al. 2016; Schulz et al. 2006). Although in many cases a medicinal plant is considered for one special therapeutic component, such as aspirin. In some cases, herbal drugs have several bioactive components that can exert the synergistic effects and also positively modify the absorption and metabolism of therapeutic ingredients or ameliorate their adverse effect (Williamson 2001). Moreover, some herbal drugs are able to affect the different diseases or several targets of the pathophysiology of one disease which is useful for depression as a complex disease and is also highly comorbid with other disorders (Sarris et al. 2011).

Persian herbal remedies have been employed over thousands of years to cure mental disorders (Ayati et al. 2020). There are several medicinal plants in traditional Persian medicine (TPM) used to treatment of

depression (Shahmansouri et al. 2014; Kolouri et al. 2016; Bazrafshan et al. 2020). Poly-herbal therapy is a commonly used strategy in traditional medicine such as Chinese and Persian (Che et al. 2013; Zakerin et al. 2019). Psycodigest is a new poly-herbal formulation used by Iranian people to treat various mental disorders like depression and anxiety in the last decade. This product is made of nine Persian herbal drugs; *Aloysia triphylla*, *Citrus aurantium*, *Echium amoneum*, *Lavandula angustifolia*, *Melissa officinalis*, *Salix aegyptiaca*, *Valeriana officinalis*, *Viola odorata*, and *Cinnamomum zeylanicum*. Although the experimental and clinical studies have reported the antidepressant effects of these herbal drugs in alone (akhondzadeh et al. 2003; Sayyah et al. 2003; Shakeri et al. 2016; Karim et al. 2018; Aryanezhad et al. 2020; Farahbakhsh et al. 2019), there is no the documented report about the efficacy of the combination of them against depression. In this study, we investigated the effect of Psycodigest on CUMS-induced depressive mice.

The chronic unpredictable mild stress (CUMS) is a widespread well-established animal model of depression which commonly used for evaluating the effect of antidepressants on depressive like-behaviors in the mouse. CUMS mimics several main symptoms of human depression-like anhedonia (Li et al. 2010). The anxiety and depression-like behaviors in mice were tested by sucrose preference test (SPT), tail suspension test (TST), forced swimming test (FST), and elevated plus maze test (EPM). Moreover the serum level of Cortisol was measured. Also, the activity of the Superoxidase Dismutase (SOD) in homogenates of tissue collected from the CUMS-mice was analyzed. For safety assessment of Psycodigest, the biochemical, hematological and parameters were examined.

Material And Methods

1. Animal

Adult male NMRI mice (20–25 g, Pasteur Institute of Iran (Amol), n =10) were used in this study. The animals were housed in standard polypropylene cages in a room with controlled temperature (23 ± 2.0 °C) and 12 h light/dark cycle (6:00–18:00). They were fed ad libitum with rodent's chow and free access to drinking water. Animals were randomly divided into different experimental groups. All experiments were conducted during the light phase according to guidelines of Institutional Animals Ethics Committee of Amol University of special modern technologies (Authorization code Ir.ausmt.rec.1399.10.20. 9911) in a way to minimize the number of animals and suffering.

2. Chronic unpredictable mild stress (CUMS) procedure

CUMS test was performed based on the previous methods (Mao et al. 2009) and had been slightly modified (Table1). Mice in CUMS groups were exposed to several mild stressors including 1) food and 2) water deprivation for 24 h, 3) 1 h exposure to an empty bottle 4) reversed light/dark for 24 h, 5) cage tilting 45° for 6 h, (6) keep the cage wet for 24 h (with water 200 mL/100 g sawdust bedding) 7) physically restraint for 2 h, and (8) exposure to a foreign object (e.g., a piece of plastic) for 24 h, These stressors were randomly scheduled over one week and repeated throughout the 4-week experiment (Table 1). Non-stressed mice in the control group had free access to water and food. After 4 weeks of CUMS stimulation, the sucrose preference of mice was tested to determine the success of CUMS modeling.

3. Drugs and administration

The traditional compound of nine Iranian herbal drugs, Psycodigest, was provided from Parsiteb Kohan Asak Company (health certification code: 94505:A61K00/36). Fluoxetine (Flu) and Bupropion (Bu) were kindly provided from Abidi Company (Iran). The CUMS-mice randomly divided to 5 groups (n=10 per group) included: 1) CUMS (no injection) 2) CUMS + Vehicle (distilled water) 3) CUMS + Psycodigest (0.23ml /mice) 4) CUMS + Flu (20mg/kg) 5) CUMS + Bup (15mg/kg) and 6) Non-stressed mice as control group. All injections were performed daily by intra-gastric gavage in a volume of 10 ml/kg body weight for 4 weeks. CUMS procedure followed during treatment. The dosage of Flu and Bup obtained from previous studies (Yi et al., 2013) and the volume of concentration and volume of the gavage administration of Psycodigest determined based on human and mouse dose conversion formula (Liu et al. 2019).

Behavioral test

The sucrose preference test (SPT)

At the end of treatment, the Sucrose preference test was performed as described previously (Mao et al., 2009). On day 1, two bottles of an equal volume of 1% sucrose solution (w/v) were placed on each cage of mice. On day 2, one of the bottles was replaced with water. On day 3, mice were deprived of food and water. On day 4, each mouse was placed in a single cage with food and one bottle of 1% sucrose solution, and one bottle of fresh water. On day 5, two bottles were weighted for the calculation of sucrose and water consumption by mice. The percentage of sucrose preference was determined with the following formula:

$$\text{Sucrose preference} = \frac{\text{sucrose consumption}}{(\text{sucrose consumption} + \text{water consumption})} \times 100\%$$

Elevated plus maze (EPM)

This instrument has 2 open and 2 closed arms (30 cm long, 5 cm wide, and 15 cm height on both sides of closed arm) which are connected to a central 5 × 5 cm square platform with a height of 80cm from the floor. Mice were placed on the center square and facing the open arm of maze for 6 min (Yan et al. 2020). Entry into an arm was considered when mice placed all four paws on it. The number of entries into open and closed arms and the time spent into open arms were recorded during the last 5 min. The ratio of the time and entries into open to closed arms were calculated by the followed formula:

$$\frac{\text{The time spent in open arms}}{(\text{The total time spent in the open and closed arms})}$$

Tail suspension test (TST)

TST was carried out according to a reported procedure (Yan et al. 2020). Mice individually were suspended from 50 cm height by their tail for 6 min. the immobility times were recorded during the last 5 min.

Forced swimming test (FST)

FST was carried out based on the previous method (Mao et al. 2009). Mice were placed into a glass cylinder (diameter 10 cm, height 25 cm) filled with 15 cm of water at 25 ± 1 °C. Mice were forced to swim for 6 min

and the immobility times were recorded in the last 5 min. Mice were considered immobile when they were floated on the water without struggling.

Sample collection

After behavioral tests, the animal lightly anesthetized with ether. First, the blood samples were taken from cardiac puncture. The blood samples are divided into two parts. A part of them was centrifuged at 5000 rpm for 15 min to obtain serum and another part transferred into K2EDTA tubes (BD Microtainer, USA) for hematological analysis. Then, the brain, liver, and kidney were immediately dissected and part of them put into the liquid nitrogen tank. Finally, the serum and tissue samples stored at -80°C for further analysis. Moreover, the rest of tissues fixed in formalin for histological analyses.

Peripheral blood routine analysis

White Blood Cell (WBC), Neutrophil (NEU), Red Blood Cell (RBC), Hemoglobin (HGB), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red blood cell Distribution Width-CV (RDWcv), Red blood cell Distribution Width-SD (RDWsd), Platelet (PLT), Mean Platelet Volume (MPV), Mean Platelet Volume (PDW), Plateletcrit (PCT) were measured and analyzed by ADVIA 2120 (SIEMENS, USA).

Biochemical Analysis of serum

ALT and Creatinine were determined by the Serum Chemistry Kit (HITACHI 7180, Japan) for the evaluation of hepatotoxicity. The serum content of the Cortisol was detected through the Elisa kit of the Bioassay System (Bioassay system, CA, USA Koma Biotech, CAS# number K0331186P, CAS# number K0331230P, Seoul, Korea), the ELISA Reader (Bio-Rad 680, CA, USA). The SOD activity in the brain, kidney and liver were estimated by commercially available kits (ZellBio GmbH, Germany) following the manufacturer's guidelines.

Statistical analysis

SPSS for Windows software version 22.0 was used for statistical analysis. All data were represented as (mean $\pm$ SEM) and analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's test for comparison between all treatments. In all experiments, $P<0.05$ was considered statistically significant.

Result

Behavioral test

Psycodigest attenuated the depression-like behaviors in CUMS-induced mice but did not affect anxiety-like behavior.

In SPT, The percentage of sucrose consumption in CUMS significantly decreased compared to the control group ($P<0.001$). Treatment with Psycodigest increased the percentage of sucrose consumption compared to the vehicle as well as Fluoxetine (20mg/kg) or Bupropion (15mg/kg) (P value <0.001) (fig1).

In TST, the immobility time significantly increased in CUMS compared to the control group ($P<0.01$). Psycodigest, as well as Bupropion (15mg/kg), could significantly decrease the immobility time in CUMS mice compared to vehicle ($P<0.01$) (fig1).

In FST, the immobility time in CUMS-mice was significantly longer than the control group ($P<0.001$). In comparison to the vehicle group, the administration of Psycodigest, Fluoxetine (20mg/kg), and Bupropion (15mg/kg) led to a reduction of the immobility time with P value <0.01 , 0.05 , and 0.01 , respectively (fig1).

In EPM, the CUMS mice showed a significant decrease in the ratio of the time spent in open arms to the total time spent in open and closed arms vs control ($P<0.05$). In comparison to the vehicle, treatment with Fluoxetine (20mg/kg) and Bupropion (15mg/kg) significantly increased the ratio of the time spent in open arms ($P<0.05$ and 0.01 , respectively). But the increase in this ratio was not significantly followed by the treatment with Psycodigest (fig1).

Peripheral blood routine analysis

In the hematological test, no toxicologically significant changes related to Psycodigest, Fluoxetine (20mg/kg), and Bupropion (15mg/kg) administration were observed (Table2).

Biochemical analysis

Serum cortisol level

In comparison to the control group, the cortisol level increased in CUMS-induced mice which is significant in CUMS, vehicle, and Bupropion (15mg/kg) ($P<0.01$). The treatment with Psycodigest as well as Fluoxetine (20mg/kg) decreased the cortisol level compared to the vehicle, but it was not significant (fig2).

Serum creatinine level

The creatinine level did not change significantly in CUMS vs control group and Psycodigest and, BU15 vs vehicle group, but significantly decreased in FLU vs vehicle group ($P<0.01$) (fig2).

Serum ALT level

Although ALT was elevated in CUMS compared to the control group, it was not significant. There were no significant changes in the ALT level followed by BUP and Psycodigest treatment (fig2). The ALT level significantly decreased in FLU-treated mice vs vehicle, Psycodigest ($P<0.05$) and CUMS ($P<0.01$).

Tissue SOD activity

The SOD activity decreased in CUMS-group vs control which was significant in the brain ($P<0.01$) and kidney ($P<0.001$). Treatment with Psycodigest and drugs increased the brain SOD activity which was significant in Psycodigest ($P<0.05$) and, BU15 groups ($P<0.01$) vs vehicle. The kidney and liver SOD activity did not change followed by the administration of Psycodigest and BU15, but significantly increased by FLU ($P<0.01$ and 0.001 in liver and kidney, respectively) (fig3).

Histological evaluation

Generally, there were no remarkable lesions in the architecture of the various organs (kidney, liver, intestine and brain) that could be attributed to the effect of the administration of the tested compounds in comparison with the control group (Fig4).

Discussion

This study for the first time reported that the oral administration of Psycodigest as a combination of traditional Persian drugs for 4 weeks improved CUMS-induced depression-like behaviors as well as the antidepressant drugs, by increased sucrose preference in SPT and decreased immobility time in FST and TST. Treatment with Psycodigest had no significant effect on anxiety-like behavior in EPM, unlike antidepressant drugs. The present result showed that SPT significantly decreased in CUMS vs control group. CUMS is a well-established animal model for inducing anhedonia and depression-like behavior. So it is indicated that the present CUMS protocol correctly induced depression in mice.

The present results are in line with the previous studies which reported the antidepressant effect of some herbal ingredients of the Psycodigest. The acute orally administration of Fructus Aurantii (FA) decreased the immobility time in FST as well as fluoxetine and improved the locomotor activity in the open field test (Zhang et al. 2012, 2018). The chronic orally administration of Cinnamomum induce antidepressant and antianxiety effect (Upadhyay et al. 2016; Zada et al. 2016). The i.p. injection of the hydroalcoholic extract of Cinnamomum zeylanicum for 4 weeks in CUMS-mice increased the sucrose preference in SPT and decreased the immobility time in FST (Aryanezhad et al. 2020). Also the acute administration of water extract of Cinnamomum zeylanicum reduced the immobility time in FST (Farahbakhsh et al. 2019). Moreover, the oral administration of Cinnamaldehyde, the main component of Cinnamomum exerted an antidepressant effect in CUMS-rats (Wang et al. 2020; Yao et al. 2015). The acute i.p. injection of the flavonoids of Viola odorata had an antidepressant like-effect in FST and TST in mice (karim 2018). The animal and human study reported the antidepressant and antianxiety effects of Melissa officinalis which was similar to fluoxetine (Lin et al. 2015; Araj-Khodaei et al. 2020). The administration of the hydro-alcoholic extract of Melissa officinalis for 14 days to restraint stress-mice showed the antidepressant and anxiolytic effect in FS, TST, EPM, and Open Field test and also improved the reduced serum levels of corticosterone, decreased MDA levels, and enhanced enzymatic antioxidant activities (Ghazizadeh et al. 2020). The co-administration of Lavandula with standard antidepressant drugs such as imipramine (Akhondzadeh et al. 2003), venlafaxine (Nikfarjam et al. 2017), and citalopram (Nikfarjam et al. 2013) in the patients with major depression lowered the mean of the depressant score more than the antidepressant drugs in alone. Sayyah et al, reported that the daily administration of Echium amoenum aqueous extract for 6 weeks had a significant antidepressant effect in a patient with major depressive disorder (sayyah et al. 2006). Moreover, the 8 weeks daily receiving of Echium amoenum in major depressive patients lowered the mean of the depressant score more than citalopram (Anushiravani et al. 2019). The subacute orally treatment of methanolic and ethanolic extract of Valeriana officinalis in rat exerted the antidepressant and anxiolytic effect (Hattesoehl et al. 2008).

It was indicated that CUMS lead to HPA-axis hyperactivation and increased the HPA-axis related molecules such as cortisol or corticosterone (Wei et al. 2017; Zhao et al. 2018). According to our result, the cortisol increased significantly in CUMS mice compared to the control group. So it is confirmed that HPA-axis was hyperactivated following the present CUMS model. Like Fluoxetine, Psycodigest was not able to significantly decrease the cortisol level vs vehicle. According to these results, it could be suggested that the suppression of HPA activity is not responsible for the antidepressant function of Psycodigest. Although the previous studies were reported that the plasma cortisol level was increased in mice and rats under acute and chronic stress (Gong et al. 2015; Zhang et al. 2011; Yin et al. 2007), Cortisol is secreted insufficient in the laboratory rodents and corticosterone is considered as the main glucocorticoid in mice and rats (Touma et al. 2005). So maybe it would be better to measure corticosterone instead of cortisol. On the other hand, the previous studies reported the different results of the effects of antidepressant drugs on the cortisol level (Nandam et al. 2020; Manthey et al. 2011). The both baseline and post-dexamethasone plasma cortisol level did not change in the major depressive patients after successful treatment by fluoxetine (Vythilingam et al. 2004). Moreover, the chronic injection of Bupropion (15mg/kg) significantly increased the plasma cortisol level as well as the vehicle compared to control group. Rao et al. reported that the treatment with bupropion for 8 weeks in patients with major depression did not affect urinary cortisol level in the treatment responder and increased the urinary cortisol level in non-responders. Bupropion inhibit the dopamine reuptake transporters and lead to an increase of the concentration of synaptic dopamine (Foley et al. 2006). The CRH neurons in paraventricular nucleus receive dopaminergic fibers. Stimulation of dopaminergic receptors leads to increased CRH releasing and consequently increased glucocorticoid production (Eaton et al. 1996). So it could be suggested that the chronic administration of Bupropion are able to raise the serum concentration of cortisol by increasing the synaptic level of dopamine.

The high level of serum ALT is a specific marker of the liver damage which occurred in depressed animal (Jia et al. 2016). Present results indicated that ALT was elevated in CUMS-mice compared with the control group, but it was not significant. Although Psycodigest and antidepressant drugs ameliorated the increased ALT level induced by CUMS in comparison to vehicle and CUMS groups, only fluoxetine exerted the significant effect. Furthermore, Psycodigest did not alter the serum level of creatinine as an indicator of kidney function which increased through renal toxicity. The creatinine level decreased in mice-treated by fluoxetine that may be due to the lowering of the muscle mass caused by CUMS-procedure (Xu et al. 2012). So, the chronic administration of Psycodigest had no hepatotoxic and nephrotoxic effect.

The level of oxidative stress markers increased in depressive disorders (Balmus et al. 2016). SOD is one of the main antioxidant enzymes that its decrease reflected the oxidative stress conditions. The previous studies reported that the SOD level (Zhang et al. 2009) and activity (Bhatt et al. 2014) decreased in the liver and brain of CUMS-mice, respectively. The present results revealed that SOD activity is suppressed in the brain and kidney of the animals in CUMS group vs non-CUMS which indicated that CUMS resulted in dysfunction of the antioxidant defense. Although the chronic administration of Psycodigest did not affect the SOD activity in the liver and kidney, but significantly elevated the SOD activity in the brain as well as Bupropion vs vehicle group. So it could be suggested that the antidepressant effect of Psycodigest against CUMS is related to improve the anti-oxidative defense system in the brain.

In conclusion, this study demonstrated that the chronic administration of the new formula of the nine Persian herbal drugs is significantly able to exert the antidepressant and neuroprotective effect in CUMS-mice without any adverse effect on the blood, liver and kidney.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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Availability of data and material

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethical approval

All experiments were conducted according to guidelines of Institutional Animals Ethics Committee of Amol University of special modern technologies (Authorization code Ir.ausmt.rec.1399.10.20. 9911) in a way to minimize the number of animals and suffering.

Author contributions

HG contributed to the design of the study, supervision of experiments, writing the manuscript and manuscript critique. AA performed the biochemical tests and wrote the manuscript. BMA performed the blood and tissue sampling and wrote the manuscript. NT performed the animal experiments. HGo performed the histological evaluation.

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Tables

Table1. CUMS procedure.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Physically restraint	9-11						
Reversed light/dark	9→	9					
Exposure to an empty bottle		9→10					
Cage tilting 45°		9→15					
Water deprivation			9→	9			9→
Reversed light/dark			9→	9			
Food deprivation				9→	9		9→
Keep the cage wet				9→	9		
Cage tilting 45° for 6 h					9:30→14:30		
Exposure to an empty bottle						9→10	
Exposure to a foreign object						10→	10

Table2. Effect of Psycodigest treatment on the Peripheral blood parameters in CUMS-exposed mice. The

data represented the values of mean $\pm$ SEM. Control; no-CUMS and no treatment. CUMS; Chronic unpredictable mild stress. Vehicle; normal saline. Flu20; Fluoxetine (20mg/kg). Bup15; Bupropion (15mg/kg).

	Control	CUMS	Vehicle	Extract	Flu20	Bu15
WBC 10 ³ /uL	4.7±1.67	6.42±1.09	5.27±1.74	7.64±1.18	6.8±0.48	5.9±0.91
HGB 10 ⁶ /uL	10.67±3.2	14.3±0.38	10.07±2.97	13.46±0.4	14.07±0.23	12.72±0.32
RBC	8.05±0.69	9.31±0.22	6.54±1.88	8.58±0.24	9.17±0.11	8.57±0.07
HTC%	38.05±3.39	41.8±1.02	30.82±9	41.16±1.41	40.85±0.5	38.36±0.68
MCV fL	46.57±1.02	44.95±0.32	46.55±0.91	48.02±0.76	44.62±0.87	44.76±0.51
MCH pg	15.75±0.53	15.3±0.04	15±0.5	15.62±0.18	15.3±0.21	14.8±0.24
MCHC g/dL	34±1.38	34.17±0.31	32.4±0.8	32.7±0.7	34.4±0.8	33.13±0.64
RDWcv %	18.35±0.34	17.85±0.49	18±0.61	20±0.7	18.8±0.87	18.46±0.29
RDWsd	28.87±1.04	27.8±0.54	28.45±0.94	31.26± 0.78	27.8±1.53	27.26±0.56

fL						
PLT	506.25±	445.5±118.85	374.25±140.5	485.8±129.31	482±78.83	351±225.65
10 ³ /uL	157.3					
MPV	7.22±	6.75±	6.75±	7.22±0.31	6.97±0.28	6.5±0.3
fL	0.4	0.27	0.27			
PDW	14.62±0.2	15.02±0.2	15.02 ± 0.2	14.96±0.24	14.87±0.3	14.6±0.2
PCT	0.35± 0.1	0.26 ± 0.1	0.26 ± 0.1	0. 34±0.091	0.32±0.05	0.22±0.15
%						

Figures

Figure 1

Effect of Psychodigest treatment on CUMS-induced depression like behavior in mice. A) the percentage of sucrose consumption in sucrose preference test (SPT), B) the immobility time in tail suspension test (TST), C) the immobility time in forced swim test (FST), D) the ratio of time spent in open arms of elevated plus maze (EPM). The data represented the values of mean $\pm$ SEM. Control; no-CUMS and no treatment. CUMS; Chronic unpredictable mild stress. Vehicle; normal saline. FLU20; Fluoxetine (20mg/kg). BUP15; Bupropion (15mg/kg). #, ## and ### P value<0.05, <0.01, and <0.001 respectively vs control. *, ** and *** P value<0.05, <0.01, and <0.001 respectively vs Vehicle.

Figure 2

Effect of Psycodigest treatment on the serum level of A) Cortisol, B) ALT and C) Creatinine in CUMS-exposed mice. The data represented the values of mean±SEM. Control; no-CUMS and no treatment. CUMS; Chronic unpredictable mild stress. Vehicle; normal saline. FLU20; Fluoxetine (20mg/kg). BUP15; Bupropion (15mg/kg). ## P value <0.01 vs Control, * P value <0.05 vs Vehicle and Psycodigest, ** P value <0.01 vs vehicle.

Figure 3

Effect of Psycodigest treatment on superoxide dismutase (SOD) activity of A) Brain, B) Kidney, C) Liver in CUMS-exposed mice. The data represented the values of mean $\pm$ SEM. Control; no-CUMS and no treatment. CUMS; Chronic unpredictable mild stress. Vehicle; normal saline. FLU20; Fluoxetine (20mg/kg). BUP15; Bupropion (15mg/kg). ## and ### P value<0.01 and <0.001 respectively vs control. *, ** and *** P value<0.05, <0.01, and <0.001 respectively vs Vehicle.

Figure 4

The histological evaluation of the Psycodigest effect in CUMS-exposed mice. H&E stained section of the liver, kidney, intestine and brain from the different groups, (A, B, C and D): control group, (E, F, G and H): Vehicle, (I, J, K and L): Flu 20, (M, N, O, and P): Bu 15, (Q, R, S, and T): CUMS, (U, V, W, and X): Psycodigest (Scale bar: 100 µm).