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Antistress Effect of Arnica montana Extracted Oil Against Chronic Unpredictable Stress Induced Depression in Rats: Behavioral, Histological and Genetic Study

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DMC and TME, isolated from Arnica montana oil, exhibit significant therapeutic effects against depressive behaviors induced by Chronic Unpredictable Stress (CUS) in experimental animals. These compounds influence monoamine neurotransmitter metabolism, helping to restore balance in stress-affected brain functions. Additionally, they mitigate hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, a key driver of stress responses, and improve structural deficits in hippocampal tissue caused by CUS. Notably, DMC and TME effectively control Brain-Derived Neurotrophic Factor (BDNF) gene expression in the animal hippocampus, a critical marker for neuroplasticity and resilience against stress-induced damage. By modulating the HPA axis and enhancing BDNF expression, these compounds alleviate depressive behaviors in CUS-affected rats, showcasing their potential for stress-related mental health disorders. Collectively, DMC and TME demonstrate their ability to attenuate CUS-induced depressive-like behaviors, providing promising insights into novel therapeutic approaches for managing depression and stress-related neuropsychiatric disorders.

Keywords: Arnica Montana; dimethoxy-p-cymene (DMC); thymol methyl ether (TME); Panax quinquefolium; chronic unpredictable stress (CUS).

1. Introduction

Chronic Unpredictable Stress (CUS) is a widely used experimental model for inducing stress in animals to mimic the effects of chronic stress in humans. This model involves exposing animals to a series of unpredictable and variable stressors, such as irregular light cycles, noise, food or water deprivation, and physical restraints. CUS is designed to prevent the animals from being inhabited to stress, thereby creating a sustained stress response. It is commonly employed in research to study stress-related disorders, including depression. CUS is particularly useful for evaluating potential treatments and understanding the physiological, behavioral, and molecular mechanisms of chronic stress. CUS profoundly impacts the hippocampus, causing changes in cortisol levels and Brain-Derived Neurotrophic Factor (BDNF) gene[1]. BDNF has an important function to play in the endurance of neuronal health and plasticity. Moreover, Hippocampus are involved in memory and emotion and after applying the CUS, BDNF expression are often significantly reduced in the hippocampus. This decrease in BDNF is associated with impaired synaptic function, neuronal atrophy, and depressive-like behaviors. According to reported literatures that natural products are very important and have gained significant attention as potential antidepressants due to their ability to modulate neurotransmitters and neurotrophic factors with lesser side effects[2]. Furthermore, these bioactive compounds isolated from plants, fungi, or marine organisms[3], interact with key pathways implicated in CUS, such as the monoaminergic systems and axis of HPA.

These natural products are not only promising for treating depression but also serve as templates for developing novel therapeutic agents. However, further clinical studies are essential to establish their efficacy and safety comprehensively[4]. In this present study we analyzed behavioral studies to comprehend the effectiveness of DMC & TME isolated from A montana and for this we performed different CUS models including depression model like FST and TST. We performed histopathological section and BDNF gene expression of different hippocampus regions to decipher the anti-stress role of DMC & TME.

2. MATERIALS AND METHODS

Chemicals and Reagents

All chemicals necessary for the experiment were purchased from sigma and the other reagents were procured from local market.

Animals

Animals were taken from our IAEC approved institution (1205/PO/Re/S/08/CPCSEA) weighing 200–260 gms. and were housed in a good ventilated, contaminated microbial free animal experimental room. Animals were kept in dark and light regulated chambers. The temperature was 24 ± 2°C and 35% to 75 % humidity. Animals were fed with an approved free access water and balanced diet.

Experimental Protocol

Four groups were taken first control, chronic unpredictable stress (CUS), DMC (40 g/kg), and TME (50 g/kg) and standard drug fluoxetine (20 mg/kg). Animals used in CUS and control group treated with 1% CMC as vehicle. We were deprived of the animals with water and food

as suggested by the protocol.

CUS were exposed to the experimental animals in a random manner hence the animals were unaware of the stressor.

BEHAVIORAL STUDIES

Rotarod experiment in mice

We also performed rotarod experiments to assess neuromuscular coordination in animals[5]. To analyze the muscular defect, we have an instrument named Rotamex 4/8 purchased from Columbus Instruments. In this instrument a moving rod covered with polypropylene foam for better grip. The moving rod (at 8rpm) is 16cm above the ground. Animals were acclimatized on the rotarod for 2 minutes for 2 consecutive days. After that animals were test after and before the treatment to check the efficacy of compounds.

Tail Suspension Test

Animals were suspended to 60 cm above the normal surface with strong tape followed by 1 min adaptation and immobility duration was recorded for 5 min according to Steru et al., 1985[6].

The animals were suspended 60 cm above a stable surface using strong adhesive tape, ensuring a secure position for observation. Following a 1-minute adaptation phase, the duration of immobility was measured for 5 minutes. This method, as described by Steru et al. (1985)[6], is widely used to assess behavioral responses related to stress or depression-like states. Immobility, defined as the absence of purposeful movement, is interpreted as a sign of behavioral despair. The test provides valuable insights into the efficacy of pharmacological or therapeutic interventions targeting stress-related conditions. By standardizing conditions such as suspension height and observation time, this procedure ensures reliable and reproducible results for understanding animal behavior in response to experimental manipulations.

Forced Swim Test

According to Porsolt et al. (1977) we performed the Forced Swim Test, and animals were placed in a 20 cm height and 11 cm in diameter glass cylinders filled with lukewarm water up to the half level of the cylinder jar[7]. Animals were allowed to swim in water for 5 minutes, after that kept in their respective cages. Next day we repeated the same experiment with 1 min acclimatization time and recorded their total mobility time.

Staining of the different region of hippocampus CA1 and Dentate Gyrus (DG)

Animals tissue sample was taken and kept in 4% paraformaldehyde for 24 hrs. After that samples were removed from para formaldehyde and embedded in paraffin to section them on slides. Moreover, staining samples were washed with xylene and 10-90 % ethanol for a short time duration. Then, sections were stained with hematoxylin with follow the staining protocol. Finally, dehydrated and the good quality sections were chosen and fixed with Canada balsam and observed under a microscope.

Statistical analysis.

The statistical data were analyzed by using prism 5.0 and the significance was determined by

ANOVA and Student's T-test followed by Dunnett.

3. RESULTS

Depression models

Effects of DMC and TME at (50 mg/kg body p.o.) were studied in the tail suspension test of depression model whereas a lower dose of DMC and TME showing no significant effect on immobility time when compared with the vehicle-treated groups. In addition, DMC and TME (50mg/kg) showing decrease the immobility time whereas reference drug fluoxetine (20mg/kg, p.o.), reduced by 91%.

Histopathological alterations

Figure 1 is the section of hippocampus of rat brain, depicting the CA1 and DG region of normal animal. Also, we all know that in CUS the most affected area is hippocampus and especially CA1 & DG.

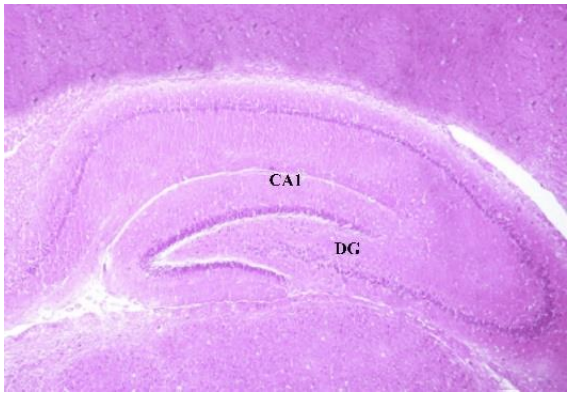


Figure 1: HE stains rat brain: deciphering the CA1 and DG region of Hippocampus.

Figure 1 depicts the CA1 region of hippocampus in this region we analyzed the three-group control, DMC & TME treated and reference groups. When we compare these three, we found no effect in vehicle control groups, although in only CUS exposure group a clear damage in the neuronal layers and which was reverted to normal after the treatment of DMC & TME. After the treatment of herbal compound DMC, it normalizes the CUS impact on CA1 where TME is not so significant. Whereas in reference fluoxetine group it normalized near to normal.

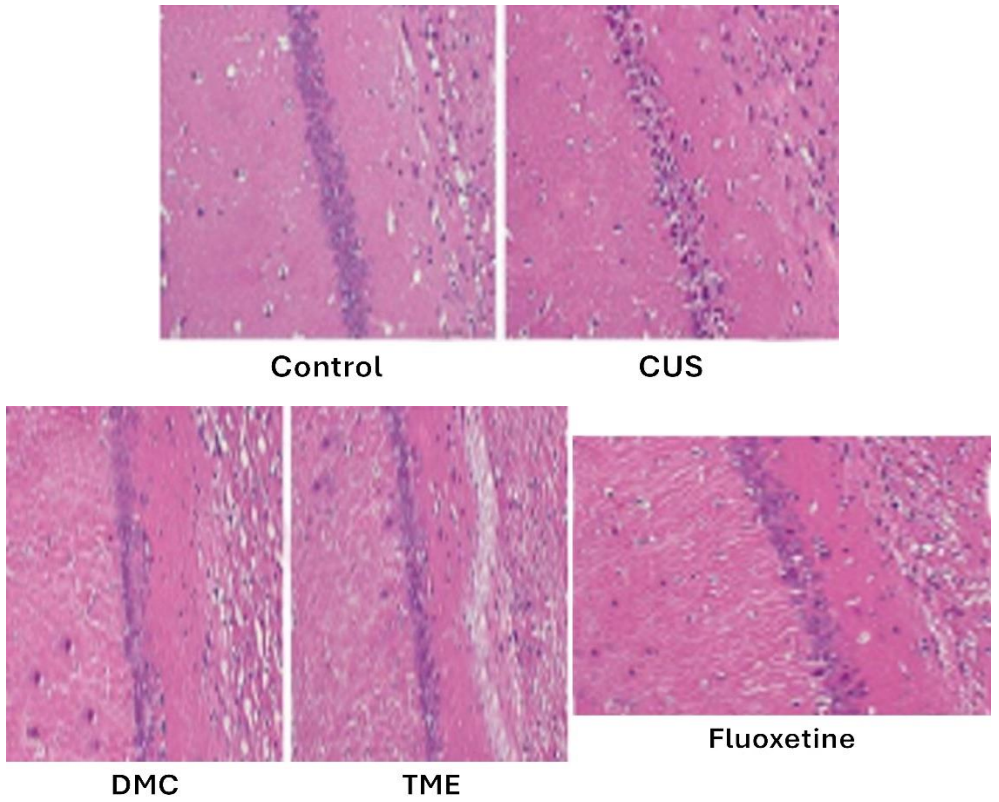
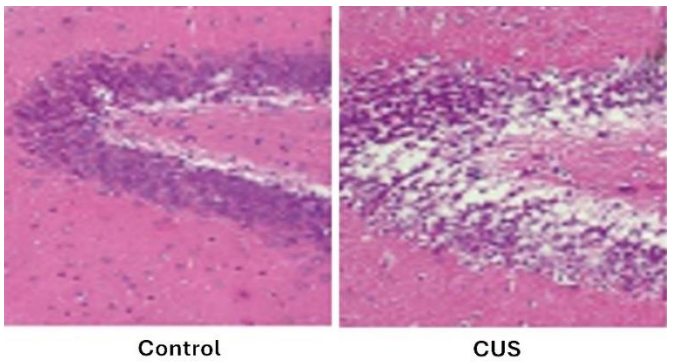


Figure 2: Staining image of CA1 hippocampal regions of Control, CUS groups, DMC & TME treated groups. Also reference drug Fluoxetine revert to normal level.

Figure 2. depicts the DG region of hippocampus in this region we analyzed the three-group control, DMC & TME and reference groups. When we compare these three, we found no effect in vehicle control groups, although in only CUS exposure group a clear damage in the neuronal layers and which was reverted to normal after the treatment of DMC & TME. After the treatment of herbal compound DMC, it normalizes the CUS impact on DG where TME is not so significant. Whereas in reference fluoxetine group it normalized near to normal.



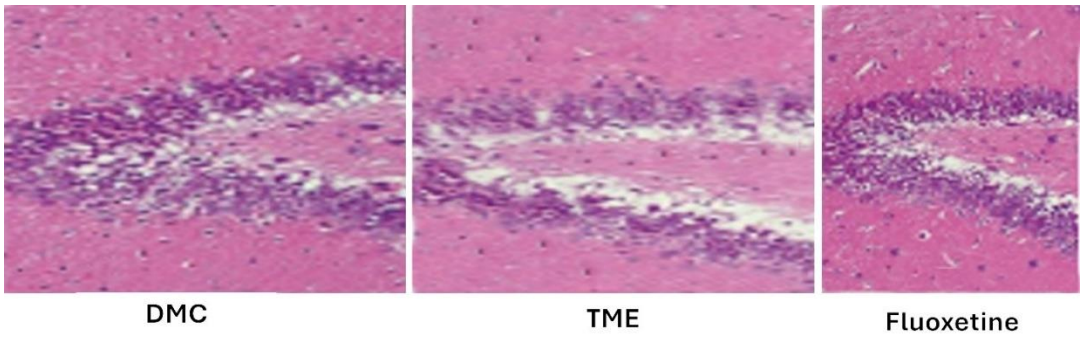


Figure 3: Staining image of DG hippocampal regions of treatment groups Control and CUS DMC & TME and reference drug fluoxetine

RT-PCR gene expression

We all know that in CUS, BDNF gene expression was decreased and if any herbal compound has potential to restore it then it has anti stress and anti-depressive potential. Here in this study, we calculate delta-delta CT value of the BDNF gene expression after the treatment of herbal compound DMC & TME, in control and CUS group. We found higher CT value in CUS in comparison of control and which were normalized by the treatment of herbal compounds we were calculating the ameliorative effect of DMC & TME (Figure 4).

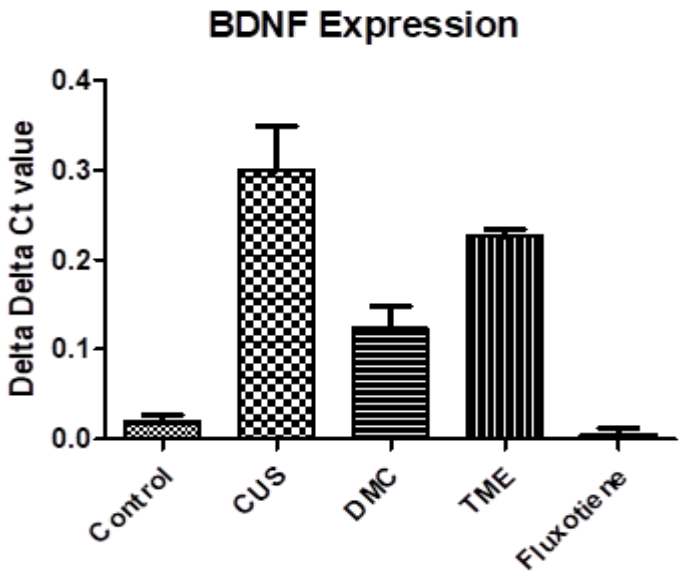


Figure 4: Delta-delta CT value of BDNF gene found lower in control, higher in CUS which were normalized by Herbal compounds DMC & TME, whereas reference drug fluoxetine lowers the CT value.

We also performed Rotarod test of DMC & TME treated rats to understand their any neuromuscular defect raised by the treatment and we found that there is no difference in herbal

compounds treated group and in control. They both spend 120sec in the rotating rod, without falling even once. This confirms the finding that the extract had no motor impairment

4. CONCLUSION

CUS causes many different diseases like ulcer[8], Diabetes[9] depression; sleep disorder[10] and many more. Moreover, depression is the most renowned disease, and it may cover all over the globe so there is an urgent need for a new and novel drug. Hence the herbal isolated compounds are more in demand that may have antidepressant effects and might be used as a therapeutic agent. In this study we aimed to analyze the behavioral effects of the DMC & TME isolated from A montana. Also, we comprehend the antidepressant effects of herbal compounds.

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