Studying anti-inflammatory action of Cordia *myxa* fruit extract on induced chronic stress model in male rats and expected improvement in depression treatment in humans

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Abstract

Depression is one of the most common phsychiatric diseases that treated with variety of medications that have significant problems to human health and increased suicide rates. Herbal medications have recently gained a lot of interest in the treatment of depression due to their safety, efficacy, and cost-effectiveness, so this study was aimed to evaluate effects of *C.Myxa* fruit extract on the model of chronic unpredictable stress (CUS) and the proinflammatory cytokine IL-6 in male rat's brains. Therefore, sixty male rats were divided into six groups. Group1 (control) was neither exposed to CUMS nor received any treatment while group 2 was exposed to CUMS for 24 days with normal saline treatment for 14 days, group 3 was exposed to CUMS for 24 days and received 10 mg/kg fluoxetine daily on day 10 for 14 days, and group 4, 5 and 6 were exposed to CUMS for 24 days and received *C.Myxa* extract (125,250 and 500 mg/kg respectively) on day 10 for 14 days. The antidepressant effect of fluoxetine and *C.Myxa* extract were evaluated by using sucrose preference test and open field test. At the end of the experiments, animals were sacrificed by decapitation, and interleukin 6 level determined by enzyme linked immunosorbent assays kit (Elisa) in rat's brain tissues. in groups 3, 4, 5 and 6, the means of sucrose preference index (SPI) on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of SPI on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day10. In group 2, the mean of SPI significantly decreased (*P-value* <0.05) as compared with group 1, while in groups 3, 4, 5, and 6 the means of SPI significantly increased (*P-value* <0.05) as compared with group 2 on day 25. Open field test (OFT) showed that in groups 2, 3, 4, 5 and 6, the means of Rearing frequencies on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of Rearing frequencies on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day10. In group 2, the mean of Rearing frequencies significantly decreased (*P-value* <0.05) as compared with group 1, while in groups 3, 4, 5, and 6 the means of Rearing frequencies, significantly increased (*P-value* <0.05) as compared with group 2 on day 25'

In terms of the Interleukin-6 levels, The means of the IL-6 concentrations in brain tissue of group 2 significantly increased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of the IL-6 concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group 2. In conclusion, *C. Myxa* has an antidepressant-like activity and reversed the stress-induced IL-6 higher levels.

Key words: Chronic unpredictable stress; Depression; cordia myxa; Interleukin-6; open field test, sucrose preference test

Introduction

Depression is a serious and widespread mental condition that affects around 350 million people worldwide of all ages. It is the fourth most common cause of incapacity [1]. At some time in their lives, one out of every five women and one out of every eight males will experience a major depressive episode [2]. Sadness, lack of interest or pleasure, feelings of guilt or poor self-worth, sleep or eating disruptions, fatigue, and reduced attention are all signs of depression. Suicide and an increased chance of mortality are possible outcomes of depression in its most severe form. It's often a long-term problem [3]. It has a detrimental influence on people's quality of life and performance even in its mildest form [4].

Because depression is such a complex condition with various possible causes, the precise process by which it manifests itself is yet unclear. Significant differences in the hypothalamic–hypophyseal–adrenal axis, as well as insufficient levels of monoamine neurotransmitters, appear to be produced by genetic, epigenetic, and environmental factors. Monoamine levels have a

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role in the pathophysiology of depression, as evidenced by reduced Dopamine DA levels in patients with severe depression and lower cerebrospinal fluid monoamine metabolite levels in those who attempted suicide while depressed. Furthermore, stimulation of the serotonergic system can increase dopamine release in those who are depressed. As a result, the dopaminergic and serotonergic systems may interact [5].

TCAs, MAOIs, and other antidepressants are categorized into numerous groups. SSRIs. NDRIs, SNRIs. Atypical antidepressants, selective NRIs, and others.

Selective serotonin reuptake inhibitors are the first-line antidepressants for children and adolescents with depression. Fluoxetine has been approved by the Food and Drug Administration (FDA) for children aged 8 and up. Escitalopram was approved for use in people aged 12 and up. The medicine fluoxetine has the most evidence for use in children with depression. While sertraline and escitalopram are considered first-line antidepressants for people suffering from severe depression [6].

Herbal medications have received a lot of attention because of their potential to treat mental illnesses. There has been an increase in their usage in depression therapies because their high effectiveness, safety , and inexpensive cost [5]. So this study was aimed to evaluate the antidepressant effects of the ethanolic extract of the *Cordia Myxa fruit* in male rats and to define the relationship between the depression and inflammation in male rats especially in brain tissue and the effects of fruit extract of *Cordia myxa* on these mediators.

Materials and methods:

Animals

There were sixty male albino rats in this research, all of them were adults. They weighed between 185 and 245 grams. The rats were kept in the Medical College/University of Babylon's Animal House at a temperature of around 25°C, with a 14-hour light/ten-hour dark cycle and unrestricted access to water and food. They were housed in 12 cages, each with five rats. Following a three-week adaptation period, the animals were randomly divided into six groups for the experiment planning.

Plant preparation

In July 2021, C. myxa dried fruit was acquired in Diyala, Iraq. The plant was authorized as C.myxa with the help of the University of Al-qasim green/ faculty of agriculture/medicinal plant department, according to document no. 3115 dated November 18, 2021. The dried fruits of Cordia Myxa were ground into powder using a mechanical grinder and stored at 4 degrees Celsius.

Fruit powder was extracted using ethanol maceration. The powdered fruits were macerated for 72 hours at room temperature in 70% (v/v) ethanol (5:10 w/v). After then, it was shook for four hours. Buchner funnel and Whatman filter paper No. 1 were used to filter the mixture. A rotating evaporator at 40°C condensed the resultant extract under pressure. The extract was kept in the fridge. [7]

General Experimental Procedure

1. On days 0, 10, and 25, each animal underwent behavioral test SPT, OFT.
2. The animals in group 1 (control) did not receive any medication and were not stressed.
3. For 24 days, each animal in groups two, three, four, five and six was exposed to chronic unpredictable mild stress (CUS).
4. Each rat in group two was given 0.2 milliliters of normal saline by oral gavage for 14 days starting from the tenth day of CUS without any treatment.
5. For 14 days, each animal in group 3 given fluoxetine treatment 10mg/kg by oral gavaging.
6. For fourteen days, each rat in groups 4, 5, and 6 was given a daily dose of C.myxa fruit extract of 125 mg/ kg, 250 mg /kg, and 500 mg/ kg, respectively by oral gavage. [8]

Chronic unpredictable mild stress (CUMS)

The Katz method, with minor modifications, was used to induce chronic stress. This protocol was chosen since it has previously been used to generate anxiety in animals. The animals in stress subject to CUMS as shown in table 1. [9]

Table 1 chronic unpredictable mild stress CUMS protocol

Day	CUS protocol
1	15 min forced swim (20 °C), crowded cage
2	12 h cage tilting (45 °C), 1 h restraint
3	reversal of the light/dark cycle
4	12 h wet bedding, crowded cage
5	24 h food deprivation, 1 h cage ratioton
6	12 h cage tilting (45 °C), tail pinch.
7	1 h cage rotation, 1 h cold room isolation
8	reversal of the light/dark cycle, tail pinch
9	24 h water and food deprivation
10	12 h cage tilting (45°C), 15 min forced swim (20 °C)
11	1 h restraint , 24 h water deprivation
12	reversal of the light/dark cycle, 24 h food deprivation
13	12 h cage tilting (45 °C)
14	24 h water deprivation, 1 h restraint
15	12 h wet bedding, 12 h cage tilting (45 °C)
16	1 h cage rotation, reversal of the light/dark cycle
17	1 h restraint, crowded cage
18	12 h wet bedding, 12 h cage tilting (45 °C)
19	reversal of the light/dark cycle, tail pinch
20	15 min forced swim (20 °C), 1 h cold room isolation
21	1 h cage rotation, crowded cage
22	reversal of the light/dark cycle , tail pinch
23	24 h food and water deprivation
24	12 h cage tilting (45 °C), crowded cage

Sucrose preference test (SPT)

SPT assesses anhedonia in rats as one of the fundamental symptoms of depression. Rats were initially trained to drink a 1 percent (w/v) sucrose solution for 72 hours without water access. Then 1% sucrose in one bottle was replaced with tap water for the next 24 h. Following training, rats were subjected to a one-hour test session after 24 hours of food and water restriction. Rats were taken from their home cages and placed separately into test cages before to the test. Each rat was given two bottles containing either tap water or a 1 percent sucrose solution on either the left or right side of the test room. After refilling the bottles, and the rats were free to drink water and sucrose solution for 1 hour. Sucrose preference (%) was determined as sucrose intake (g) divided by total liquid consumption (g) multiplied by 100 during the 1-h test. The test was done on day 0 (for baseline data), 10, and 25. [10]

Open-field test

According to Starac [11], this wooden box (100 x 100cm) was built by a researcher and consists of a square floor divided into 100 equal squares by thin white lines. To prevent escape, the equipment consists of an arena surrounded by high walls. The number of square crossings, rearing, and time spent moving are utilized to evaluate the rat's activity throughout the test period. Crossings and rearings behaviors are used to assess hyperactivity in the open-field apparatus. The total number of square crossings throughout the test time is referred to as crossings, and it is used to determine the animals' locomotor activity. The total number of upright postures taken by the rat with the intention of investigating during the test time is referred to as rearings. Stereotypy refers to animals' repeated actions, which are more common in animals with brain abnormalities. Grooming and sniffing habits might be used to assess it. Grooming refers to the total time spent grooming during the test period, whereas sniffing refers to the total time spent sniffing during the test period. In order to quantify anxiety-like behavior, the quantity of time spent in the middle of the arena was assessed. This test is based on rats exploring an open field on their own. When rats are anxious, they will naturally seek shelter towards the margins of an open field's perimeter. As a result, less time spent in the center of the arena reflects higher anxiety-like behavior[12].

Tissues samples preparations

On the twenty-fifth day, the animals were beheaded one day following the final treatment. The brains were retrieved after slicing a skull from the foramen magnum posteriorly. After the olfactory pulps and cerebellum were cut, the brain was gently removed from the skull. After being cleaned in phosphate buffer saline solution, the mid and forebrain were dissected and weighted. It was kept in a sterilized eppendorf tube before being deep frozen on dry ice at -20 C^0.

Rat Interleukin 6 ELISA Kit

The principle of this kit was that Rat IL-6 antibody has been pre-coated on the plate. IL-6 from the sample is introduced to the wells, where it binds to antibodies. The biotinylated Rat IL-6 Antibody is then added to the sample and binds to IL-6. The biotinylated IL-6 antibody is then bound by Streptavidin-HRP. During the washing phase after incubation, all unbound Streptavidin-HRP is rinsed away. Following that, the substrate solution is added, and the color develops in accordance to the amount of Rat IL-6 present. The process is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

Analysis of Statistics

The information was provided as a mean with standard deviation (SEM). In the statistical analysis, the post hoc test and one-way analysis of variance (ANOVA) were performed (LSD and Bonferoni). The differences were considered statistically significant if the probability p value was less than 0.05. For statistical analysis, the 23rd edition of (SPSS v24) statistics for Windows ® 10 was employed.

Results

Sucrose preference tests within the group

The mean sucrose preference index (SPI) on day 25 in group 1 increased substantially (P-value < 0.05) when compared to days 10 and 0, but the mean SPI on day 25 in group 2 declined significantly (P-value 0.05) when compared to days 0 and 10. (table 2)

On day 10, the means of SPI in groups 3, 4, 5 and 6 substantially reduced (P-value < 0.05) when compared to day 0, but the means of SPI in groups 3,4,5 and 6 considerably rose (P-value < 0.05) when compared to day 10. (Table 2)

Table 2: Comparison in the means of sucrose preference index ± SEM between groups on days 0, 10, 25.

SPT	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	79.2000±1.34825	79.1000±1.87053	76.2000±2.40740	77.5000±1.57233	80.9000±1.68292	80.7000±1.61280
Day10	78.6000±1.80862	51.4000±3.19096*	57.6000±1.79162*	57.7000±2.22136*	56.3000±2.78907*	55.8000±2.64611*
Day25	82.000±1.90904 α π	53.4000±1.70098*	74.0000±1.69312 α	74.3000±1.55671 α	78.1000±2.36385 α	80.2000±1.23108 α

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

Open Field Test (OFT) results

3.1.3.1. Frequency of Rearing behavior within the group

There were no significant differences in means of rearings on days 10 and 25 as compared to day 0 in group 1 (control group, untreated and unexposed to CUS), whereas in group 2 (untreated and exposed to CUS), the means of rearing frequency on day 25 significantly increased (*P-value* <0.05)as compared to 10 (Table 3).

In groups 2, 3, 4, 5 and 6, the means of rearing frequency on day10 significantly decreased (*P-value* <0.05) as compared with day 0, while the means of rearing frequency on day 25 of groups 3,4,5 and 6 significantly increased (*P-value* <0.05) as compared with day 10. (Table 3)

Table 3: Comparison in the means of Rearings frequency ± SEM between groups on days 0, 10, 25.

Rearings	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 0	50.2000±5.41151	59.000±2.48104	53.5000±4.21703	59.6000±5.75654	50.2000±4.18144	53.3000±4.67868
Day10	48.2000±2.69485	9.8000±1.43604*	10.0000±2.21610*	10.3000±1.81384*	11.2000±1.63843*	9.3000±1.48361*
Day25	48.0000±2.43128	8.9000±1.06927*	45.7000±5.11219 α*	35.7000±3.82695 α*	39.1000±3.47515 α*	37.6000±4.90635 α*

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (Treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

Sucrose preference tests on day 25

In group 2, the mean of SPI significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of SPI significantly increased (*P-value* <0.05) as compared with group 2. (table 4, figure 1)

Table 4: Sucrose preference tests on day 25

Test	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
SPI (Sucrose preference index)	82.000±1.90904	53.4000±1.70098£	74.0000±1.69312 β	74.3000±1.55671 β	78.1000±2.36385 β	80.2000±1.23108 β

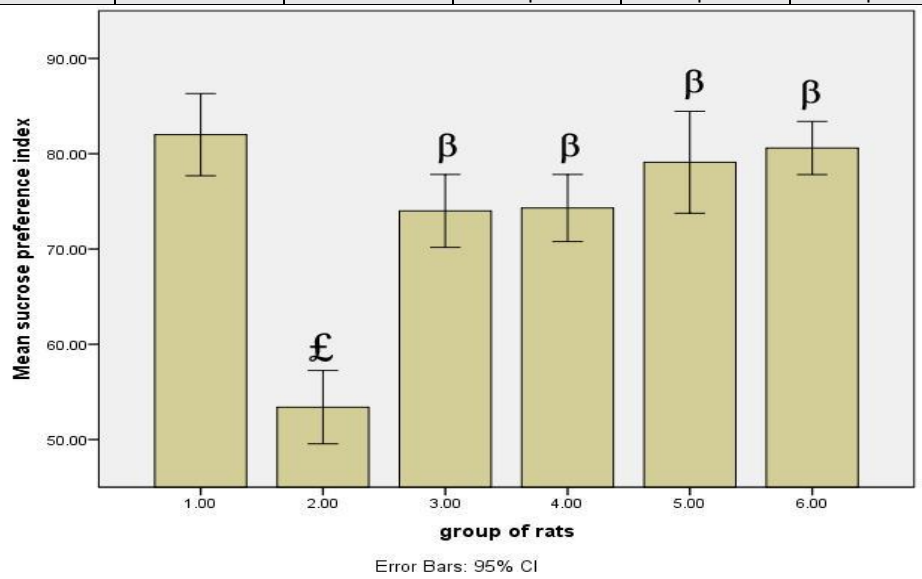

Fig 1: Means ± SEM of the SPT index of sucrose preference test on day 25 for all groups.

β = significantly increase (*P value* <0.05) as compared with group 2.

£ = significantly decrease (*P value* <0.05) as compared with group 1

Rearing frequency in open field test on day 25

In group 2, the mean of rearing frequency significantly decreased (*P-value* <0.05) as compared with group1, while in groups 3,4, 5, and 6 the means of rearing frequency significantly increased (*P-value* <0.05) as compared with group 2. In groups 4 and 6 the mean of Rearing frequency significantly decrease (*P-value* <0.05) as compared with group 1 (table 5, figure 2)

Table 5 :Rearing frequency in open field test on day 25						
Test	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
OFT (Rearing frequency)	48.0000±2.43128	08.9000±1.06927£	45.7000±5.11219 β	35.7000±3.8269 β£	39.1000±3.47515 β	37.6000±4.90635 β£

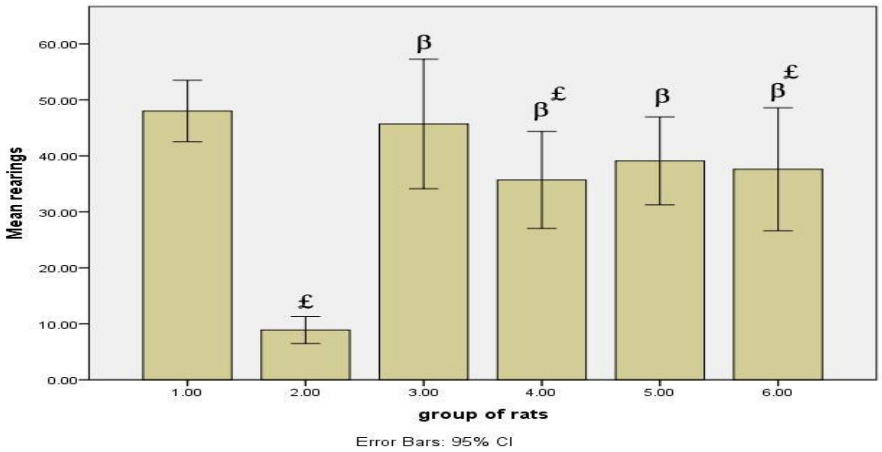

Fig 2: Means ± SEM of the rearing frequencies in open field test on day 25 for all groups.
β = significantly increase (*P value* <0.05) as compared with group 2
£ = significantly decrease (*P value* <0.05) as compared with group 1

Brain inflammatory system results

Interleukin-6 (IL-6)

The means of the IL-6 concentrations in brain tissue of group 2 significantly increased (*P-value* <0.05) as compared with group1, while in groups 3, 4, 5, and 6 the means of the IL-6 concentrations in brain tissue significantly decreased (*P-value* <0.05) as compared with group 2. (table 6, figure 3)

Table 6 Interleukin-6 (IL-6)						
Concentrations	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
IL-6 (ng/L)	8.8257±0.42629	15.5160±0.71749 π	9.3387±0.62002*	10.1393±0.42055*	9.3183±0.57823*	10.7692±0.55333*

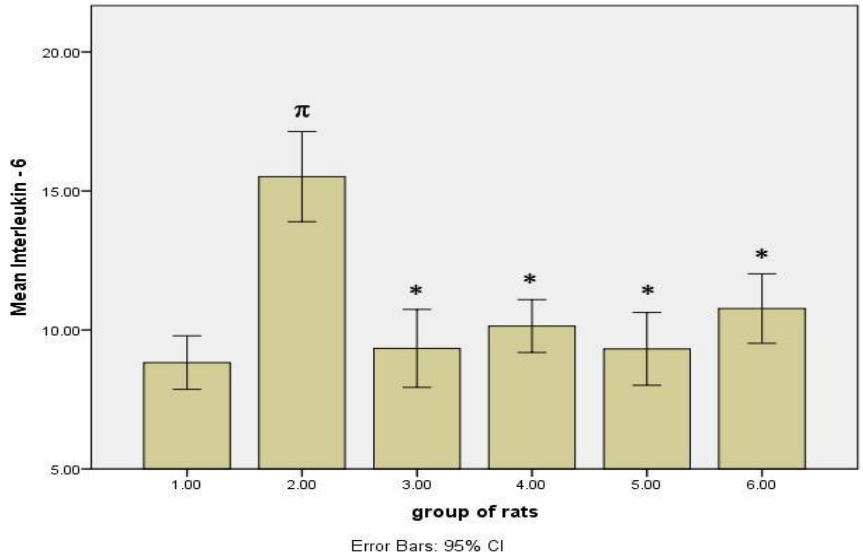

Figure 3: Means ± SEM of the Interleukin-6 concentrations (ng/L) in brain tissue for all groups.
* = significantly decrease (*P value* <0.05) as compared with group 2
π = significantly increase (*P value* <0.05) as compared with group 1

Group 1 (control group, untreated and unexposed to CUS), group 2 (untreated and exposed to CUS), group 3 (treated with 10mg/kg fluoxetine for 14 days), group 4 (treated with 125mg/kg C.Myxa extract for 14 days), group 5 (treated with 250mg/kg C.Myxa extract for 14 days), group 6 (treated with 500mg/kg C.Myxa extract for 14 days), no.of rats=10 for each group.

Discussion

Depression is a crippling illness that affects a large percentage of the population. It is a highly diverse sickness that can be caused by a multitude of factors. To test the association between stress and depression, animal models must be used, which must exclude animals that

are stress resistant in order to represent the real-life scenario in people. For example, animal models generated with maternal deprivation and chronic unpredictable stress-exposure are often employed to imitate stress experienced by humans during development and adulthood [13]. CUMS has been linked to long-term behavioral alterations that are similar to clinical depression symptoms in several studies. In fact, this study investigated the anti-depressant impact of Cordia Myxa fruit extract in rat brain tissue using behavioral tests such as the open field test and the sucrose preference test, as well as the influence on the inflammatory mediator interleukin 6. This is the only research we're aware of that links the effects of C. Myxa fruitextract on stressed male rats.

Sucrose preference index, and rates of rearings in OFT were not significantly different between animals from all groups on day 0 (baseline) in this study. When compared to baseline (day 0), there was a substantial decline in sucrose preference during SPT, and rates of rearing OFT for all groups of rats exposed to CUS after 10 days of unpredictable stress. This indicates that these animals have evolved a depression model.

The reduction in sucrose consumption is thought to represent anhedonia in animals, which is one of the two fundamental symptoms needed to diagnose a severe depressive episode in humans. [13-15]

The OFT was used as behavioral index of locomotor activity and anxiety-like behavior. This test introduced in our research to strength interpretation of SPT findings. Our results agree with Hazra findings that after exposure to 10 days of CUS, the number of rearing and line crossing in OFT significantly decreased [16]. The decreased activity in open field arena contrast with group 1 (rats not exposed to CUS) that show increased rearing, line crossing and grooming, which is driving by the interests of rats to explore a new environment. However, rats show reduced rearing, line crossing and grooming in an unfamiliar open field after CUS , which might imply a "refractory loss of interest", which is another fundamental characteristic of human severe depression [17].

It is worth noting that the results of the open field test can be also affected by several factors which are mentioned in previous studies including age, and circadian rhythm. In addition, adaptation, room temperature, humidity, lighting, noise, and even odor can affect assessment outcomes. [18]

in group 1, glucose intake was substantially higher on day 25 compared to day 0 and day 10. This might be due to glucose's rewarding impact. When feeding was limited with planned sucrose availability, the rat dopamine transporter was up-regulated in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) of the brain. Dopamine regulates dopaminergic activation of the reward region of the brain by interacting with several strong neurotransmitters such as serotonin, enkephalins, and GABA. Additionally, by activating the β3 adrenoceptors, dopamine can decrease uptake of glucose in rat white adipocytes that lack dopaminergic receptors. [19]

When compared to group 1, group 2 (untreated and exposed to CUS) had depressive-like behavior on day 25, as evidenced by significant reduction in the sucrose preference index during SPT, and lower rates of rearing (untreated and unexposed to CUS). These findings matched those of a prior research [20]. In comparison to group 2, there was significant rise in sucrose preference index during SPT, and increased frequencies of rearing OFT in groups 3,4,5, and 6. The antidepressant properties of fluoxetine and C. Myxa fruit extracts were shown by these findings.

Moreover, this study has revealed that after the treatment of rats with either fluoxetine or three concentrations of Cordia Myxa Fruit extract; antidepressant effect has been shown. That, sucrose preference index significantly increased, and increased rearing, in open field on day 25 as compared with day 10 for group 3 rats (treated with 10 mg/kg fluoxetine for 14 days from CUS beginning). This findings has been demonstrated in the previous study [5].

The antidepressant action of fluoxetine has been linked to a number of mechanisms. In people suffering from depression, activation of the serotonergic system, for example, can enhance the release of DA. Serotonergic neurons connect to the mesolimbic dopamine system and control dopamine transmission via several serotonin receptor subtypes. Serotonergic activity has been found to impact the NAcc (a prominent candidate for reward and pleasure processing). [21]

After 10 days of CUS exposure, there was a significant drop in the sucrose preference index in groups 4,5 and 6 (treated with C. Myxa extract 125 mg/kg, 250 mg/kg, and 500 mg/kg, respectively) as compared to day 0 (baseline). This illustrates a depressed state and a depression model. On day 25, there was a a significant increase in the sucrose preference index and activity in open field arena as compared to day 10 after treatment with various doses of C. Myxa for 14 days from CUS. C. Myxa was given repeatedly, and the effectiveness was equivalent to that of the clinically active antidepressant fluoxetine. C. Myxa's capacity to reverse CUS-induced anhedonia-like behavior, and increase exploratory and locomotor activity in open field test lends credence to the hypothesis that it possesses antidepressant properties.

According to previous studies, Several inflammatory and oxidative cytokines have been implicated in the mechanisms underpinning the occurrence of depression. Several lines of evidence point to this conclusion that interleukin-1 and interleukin-6 (IL-1 and IL-6) may play a role in the development of major depression as well as in antidepressant treatment therapeutic processes. [22]

In group 2 (exposed to CUS , and recieve normal saline 10 days from begining of CUS for 14 days), There was significant increases in interleukin 6 (IL-6) concentrations in brain tissues as compared with group 1 (control ; untreated and unexposed to CUS). This finding supports previous study that revealed IL-6 production was substantially greater in MDD patients than in healthy controls, and that monocytic proinflammatory cytokines have a role in MDD [23]. So, This finding reinforces the Cytokine Hypothesis. External (psychological) and internal (organic inflammatory illnesses or disorders) stresses can both trigger the inflammatory process, that proinflammatory cytokines increased and play role in development of depression. according to the cytokine theory of depression [24].

[22]. also reported the similar results that immunological dysregulation in MDD is linked to the activation of monocytic proinflammatory cytokines (ILs and TNF), as well as the suppression of both Th1 and Th2 cytokines in addition TGF-beta1 may have a role in the control of monocytic cytokines, as well as Th1 and Th2 cytokines. [25]. Our results meet previous Raison *et al.* findings that Increased plasma and CSF concentrations of a number of cytokines and their receptors, including IL-1, IL-2, IL-6, and TNF-a, are linked with immunological activation in major depression. [25]

The effects of cytokines on behavior are thought to be linked to their impacts on neurotransmitter and neuropeptide function, synaptic plasticity, and neuroendocrine functions. The effects of cytokines on neuroendocrine function in depression might be linked to their effects on the glucocorticoid receptor (GR) and signaling pathways that contribute to glucocorticoid resistance. Glucocorticoid resistance may be an adaptive mechanism that allows inflammatory healing to proceed despite glucocorticoid levels caused by stress. [26, 27]

Indeed, in animal and human studies, IL-1, IL-6, and IFN-c were found to cause behavioral changes and symptoms similar to those seen in depression, including an inability to feel pleasure, anorexia, weight loss, withdrawal from social situations, psychomotor retardation, irritability, and sleep disorders. [28]

In group 3 (exposed to CUS and received fluoxetine treatment for 14 days) , there was significant decrease in interleukin-6 (IL-6) concentrations as compared with group 2 (control; untreated and exposed to CUS). According to this finding, fluoxetine have anti-inflammatory properties and this confirm with previous researchs that shown that fluoxetine can lower the production of prostaglandin E2 and proinflammatory cytokines, as well as lessen the symptoms of major depression caused by IL-1 or LPS. [24]

Our result also supports Liu et al. findings that demonstrate anti-inflammatory activity of fluoxetine that reduces the production of IL-6, TNF-a, and nitric oxide in microglia [29]

Although the molecular mechanism by which fluoxetine exerts antiinflammatory activity is unclear, some authors indicate that fluoxetine operates by lowering gene expression as seen by lower transcription levels of mRNA of IL-6 and TNF-a . Additionally, fluoxetine may block the phosphorylation of mitogen activated protein kinase, a key signaling route for proinflammatory cytokines, as well as the activation of nuclear factor kappaB (NF-kB), a key inflammatory signaling molecule. [30]

Fluoxetine can act by inhibiting NF-kB, can reverse the polarization of modified macrophages, suggesting that fluoxetine could be a useful drug for reprogramming macrophages to favor the host in inflammatory situations. [31]

In group 4, 5, 6 (exposed to CUS and treated with three diffirent concentration of C.Myxa extract 125mg/kg, 250mg/kg, 500mg/kg respectively), there was significant decrease of interleukin-6 (IL-6) concentrations as compared with group 2 (received normal saline 14 days and exposed to CUS), So the plant extract have anti-inflammatory action as noted by Olukunle *et al.*, . Polyphenols, alkaloids and flavonoids in plants are known to exert active anti-inflammatory effects in the plant extract[32].

According to [33]study, the high polyphenolic content of C. myxa fruit may be responsible for its preventive effect against liver injury in rats that prevent inflammation[33]. An investigations on the anti-inflammatory effects of C. myxa fruit in an experimental colitis model and found that C. Myxa preparation inhibits the oxidative stress factors that lead to colitis progression, resulting in improvement in total antioxidant status, and return to normal levels. Furthermore, in terms of bioavailability, C. myxa fruit is a great provider of numerous nutrients [34, 35].

The Cordia myxa fruit's anti-inflammatory properties can be linked in part to its antioxidant properties and the restoration of trace element levels in inflamed tissues. [36]

Conclusions

This study reveals that prolonged unpredictable stress causes depressive-like behavior in rats, as well as increase levels of interleukin 6 which reversed by C. Myxa treatments in treated groups,

in addition to its antidepressant-like action as shown by behavioral tests. Additionaly, results obtained in our study were comparable to that of fluoxetine that suggest cordia myxa also have anti depressant like effects in animal models of depression.

References

1. Kessler RC, Bromet EJ. The epidemiology of depression across cultures. Annual review of public health. 2013;34:119-38. https://doi.org/10.1146/annurev-publhealth-031912-114409.
2. Munoz RF, Cuijpers P, Smit F, et al. Prevention of major depression. Annual review of clinical psychology. 2010;6:181-212. https://doi.org/10.1146/annurev-clinpsy-033109-132040.
3. Alatab S, Sepanlou SG, Ikuta K, et al. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet gastroenterology & hepatology. 2020;5(1):17-30. https://doi.org/10.1016/S2468-1253(19)30333-4.
4. Fu CM, Parahoo K. Causes of depression: perceptions among people recovering from depression. Journal of Advanced Nursing. 2009;65(1):101-9. https://doi.org/10.1111/j.1365-2648.2008.04845.x.
5. Shen M, Yang YI, Wu Y, et al. L-theanine ameliorate depressive-like behavior in a chronic unpredictable mild stress rat model via modulating the monoamine levels in limbic–cortical–striatal–pallidal–thalamic-circuit related brain regions. Phytotherapy Research. 2019;33(2):412-21. https://doi.org/10.1002/ptr.6237.
6. Mullen S. Major depressive disorder in children and adolescents. Mental Health Clinician. 2018;8(6):275-83. https://doi.org/10.9740/mhc.2018.11.275.
7. Salimimoghadam S, Ashrafi A, Kianidehkordi F, et al. Hypoglycemic, antitussive and analgesic effects of nanoparticles of Cordia myxa fruits extract. International Journal of Pharmaceutical Investigation. 2019;9(4):205-9. https://doi.org/10.5530/ijpi.2019.4.38.
8. Shahriari M, Moghadamnia D. Protective Effect of Cordia Myxa Extract on Changes in Body Weight, Serum Proteins, Albumin and Liver Histology of Adult Male Rats Induced by Cadmium Chloride Toxicity. Iranian Journal of Toxicology. 2019;13(2):43-9. https://doi.org/10.32598/IJT.13.2.575.3.
9. Amiresmaeili A, Roohollahi S, Mostafavi A, et al. Effects of oregano essential oil on brain TLR4 and TLR2 gene expression and depressive-like behavior in a rat model. Research in pharmaceutical sciences. 2018;13(2):130. https://doi.org/10.4103/1735-5362.223795.
10. Sahin Ozkartal C, Aricioglu F, Tuzun E, et al. Chronic mild stress-induced anhedonia in rats is coupled with the upregulation of inflammasome sensors: a possible involvement of NLRP1. Psychiatry and Clinical Psychopharmacology. 2018;28(3):236-44. https://doi.org/10.1080/24750573.2018.1426694.
11. Starac A. Quarter of St. Theodor in Pula. Revija Annales Series Historia et Sociologia. 2009:19-2.
12. Valvassori SS, Varela RB, Quevedo J. Animal models of mood disorders: focus on bipolar disorder and depression. Animal models for the study of human disease: Elsevier, 2017. p. 991-1001. https://doi.org/10.1016/B978-0-12-809468-6.00038-3.
13. Zhang Y, Wang Y, Lei H, et al. Optimized animal model to mimic the reality of stress-induced depression in the clinic. BMC psychiatry. 2017;17(1):1-9. https://doi.org/10.1186/s12888-017-1335-x.
14. Keute M, Demirezen M, Graf A, et al. No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS). Scientific reports. 2019;9(1):1-10. https://doi.org/10.1038/s41598-019-47961-4.
15. Ko C-H, Yen J-Y, Chen S-H, et al. Evaluation of the diagnostic criteria of Internet gaming disorder in the DSM-5 among young adults in Taiwan. Journal of psychiatric research. 2014;53:103-10. https://doi.org/10.1016/j.jpsychires.2014.02.008.
16. Hazra S, Kumar S, Saha GK, et al. Reversion of BDNF, Akt and CREB in hippocampus of chronic unpredictable stress induced rats: effects of phytochemical, Bacopa Monnieri. Psychiatry Investigation. 2017;14(1):74.
17. Tatem KS, Quinn JL, Phadke A, et al. Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. JoVE (Journal of Visualized Experiments). 2014(91):e51785. https://doi.org/10.3791/51785.
18. Liu Q, Li B, Zhu H-Y, et al. Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment. Journal of Affective Disorders. 2011;128(3):309-13. https://doi.org/10.1016/j.jad.2010.07.007.
19. Wang D, An SC, Zhang X. Prevention of chronic stress-induced depression-like behavior by inducible nitric oxide inhibitor. Neuroscience letters. 2008;433(1):59-64. https://doi.org/10.1016/j.neulet.2007.12.041.
20. Kranz GS, Kasper S, Lanzenberger R. Reward and the serotonergic system. Neuroscience. 2010;166(4):1023-35. https://doi.org/10.1016/j.neuroscience.2010.01.036.
21. Zong J, Liao X, Ren B, et al. The antidepressant effects of rosiglitazone on rats with depression induced by neuropathic pain. Life Sciences. 2018;203:315-22. https://doi.org/10.1016/j.lfs.2018.04.057.
22. Kim Y-K, Na K-S, Shin K-H, et al. Cytokine imbalance in the pathophysiology of major depressive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2007;31(5):1044-53. https://doi.org/10.1016/j.pnpbp.2007.03.004.
23. Chopra K, Kumar B, Kuhad A. Pathobiological targets of depression. Expert opinion on therapeutic targets. 2011;15(4):379-400. https://doi.org/10.1517/14728222.2011.553603.
24. Song C, Halbreich U, Han C, et al. Imbalance between pro-and anti-inflammatory cytokines, and between Th1 and Th2 cytokines in depressed patients: the effect of electroacupuncture or fluoxetine treatment. Pharmacopsychiatry. 2009;42(05):182-8. https://doi.org/10.1055/s-0029-1202263.
25. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends in immunology. 2006;27(1):24-31. https://doi.org/10.1016/j.it.2005.11.006.
26. Pace TWW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and

treatment of major depression. Brain, behavior, and immunity. 2007;21(1):9-19. https://doi.org/10.1016/j.bbi.2006.08.009.
27. Makkonen I, Kokki H, Kuikka J, et al. Effects of fluoxetine treatment on striatal dopamine transporter binding and cerebrospinal fluid insulin-like growth factor-1 in children with autism. Neuropediatrics. 2011;42(05):207-9. https://doi.org/10.1055/s-0031-1291242.
28. Klietz M-L, Kaiser HW, Machens H-G, et al. Ac c ep te d Corresponding Author : an us cr ip t Ac c ep te d us cr t," pp. 1–31. 2019.
29. Liu D, Wang Z, Liu S, et al. Anti-inflammatory effects of fluoxetine in lipopolysaccharide (LPS)-stimulated microglial cells. Neuropharmacology. 2011;61(4):592-9. https://doi.org/10.1016/j.neuropharm.2011.04.033.
30. Caiaffo V, Oliveira BDR, de Sá FB, et al. Anti-inflammatory, antiapoptotic, and antioxidant activity of fluoxetine. Pharmacology research & perspectives. 2016;4(3):e00231. https://doi.org/10.1002/prp2.231.
31. Ghosh S, Mukherjee S, Choudhury S, et al. Reactive oxygen species in the tumor niche triggers altered activation of macrophages and immunosuppression: role of fluoxetine. Cellular signalling. 2015;27(7):1398-412. https://doi.org/10.1016/j.cellsig.2015.03.013.
32. Olukunle JO, Adenubi OT, Oladele GM, et al. Studies on the anti-inflammatory and analgesic properties of Jatropha curcas leaf extract. Acta Veterinaria Brno. 2011;80(3):259-62. https://doi.org/10.2754/avb201180030259.
33. Afzal M, Obuekwe C, Khan AR, et al. Influence of Cordia myxa on chemically induced oxidative stress. Nutrition & Food Science, vol 39, no 1, pp 6–15, . 2009. https://doi.org/10.1108/00346650910930761.
34. Al-Awadi FM, Srikumar TS, Anim JT, et al. Antiinflammatory effects of Cordia myxa fruit on experimentally induced colitis in rats. Nutrition. 2001;17(5):391-6. https://doi.org/10.1016/S0899-9007(01)00517-2.
35. Paul M, Bhatia M, Raj AK, et al. Ceftriaxone-sulbactam-EDTA Susceptibility Profile of Multi-drug Resistant Gram-negative Bacterial Isolates: Experience from a Tertiary Care Teaching Hospital in Rishikesh, Uttarakhand. Journal of Natural Science, Biology and Medicine. 2020;11(2):190.
36. Dewole EA, Dewumi DF, Alabi JY, et al. Proximate and phytochemical of Cola nitida and Cola acuminata. Pakistan Journal of Biological Sciences: Pjbs. 2013;16(22):1593-6. https://doi.org/10.3923/pjbs.2013.1593.1596.