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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF BIXIN INCHRONIC UNPREDICTABLE MILD STRESS.

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Key Words

Chronic unpredictable mild stress, Oxidative stress, Bixin, Fluoxetine

ABSTRACT

Depression is a condition characterized by loss of mood and interest to activity that can lessen a person's thinking, conduct, tendencies, emotional state, and the sense of well-being. Thepurpose of this study is to evaluate the effect of bixin on depression induced by chronic unpredictable mild Stress model in mice. Bixin was extracted by the soxhlation and quantified by Mass spectroscopy, Ultraviolet Spectroscopy and Thin-layer Chromatography. Further, its effect on depression was evaluated in maleBalb/Cmice bytail suspension test, sucrose preference test andbehavioural evaluation by open field test. Bixin was administered at a dose of 50mg/kg and 100 mg/kg for a period of 21 days parallelly with stressors. Fluoxetine (20 mg/kg) wasused as standardfor a period of 21 days parallelly with stressors. Oxidative stress was assessed by superoxide dismutase, lipid peroxidation, reduced glutathione. Brain acetylcholine esterase was measured at the end of the study to assess if there was cognitive impairment due to depression. The results revealed significant alteration in behavioural, biochemical parameters upon treatment with bixin when compared to control group ($p < 0.05$) indicating the antidepressant and memory enhancement potentials of bixin.

INTRODUCTION

Depression is a persistent, prevalent disease associated with physiological, and psychological alterations and allied by increased mortality [1]. Oxidative stress is the prominent causative factor in many neuro disorders/neurodegenerative diseases includes neuropsychiatric disorders like Parkinson's, Alzheimer's and depressive disorders in the brain [2]. Earlier reports suggested that Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes of inflammatory cells which emit the reactive oxygen species, which in turn induce the oxidative stress by the abrupt production of free radicals[3,4] that are involved in dysregulation and disruption of the HPA axis [5,6]. However, in Chronic Unpredictable Mild Stress (CUMS) the animal is exposed to various kind of stressors over a particular period for several weeks to a series of the minute intensity of stressors. The result has revealed the progress in the development of alterations in the behaviour including loss of anhedonia. Further, similar behavioural changes was observed in the majority of the clinically depressed patients [7]. The brain is most vulnerable to the oxidative stressowing to the inordinate intake of oxygenwhich in turn produces excitotoxicity, high lipid content, free radicals, and insufficient antioxidant defence mechanisms[8]. Bixin a carotenoid found in the seeds of *Bixa Orellana* Linn. that helps to forage the superoxide activity and the defensive

role of carotenoids which can shield the host cells from the oxygen metabolites, thoughreactive oxygen species (ROS) remainfrequently recognised for the damaging properties in the cells, a number of biological reactions are changed by theROS[8,9]. Bixin, is found to have the major capacity of antioxidants which helps in the free radical scavenging activity, where the unforeseen generation of the free radical ischaracterised to be one of the major causes of the depression[10].[11] We also wanted to assess the effect of depression on cognition for which we estimated the levels of Acetyl cholinesterase activity in the brain (AChE) along with behavioural studies .Therefore, the current study was undertaken to explore the antioxidant potential and cognitive enhancement capabilities of Bixin by Chronic Unpredictable mild stress model in mice..

1. Materials and Methods

Male Balb/C mice (2-3 months, 25-40grams) were engaged in the current study, animals were well maintained with access to water and libitum, natural light/dark cycle for 12 hours and noise level less than 85 decibels. The experimental etiquette has been duly sanctioned by the Institutional Animal Ethics Committee (IEAC). The care of the animals stayed approved out rendering to guiding principle of CPCSEA, Ministry of Environment and Forest Government of India. (Reg no: **155/PO/Re/S/99/CPCSEA)**

2.1. Bixin Extraction

Fresh seeds of *Bixa Orellana* Lin were collected from a local vendor in Mysuru on September 2018, the seeds were freed from dust and adulterants, and stowed in dim at the room temperature. Pigments present in the pericarp of the *Bixa Orellana* Lin. Seeds are extracted by mechanical abrasion method. 50 grams of seeds were agitated with ethanol: distilled water (93:7) for 24 hours at 37 ˚C, filtered and exhaustively washed with hexane to remove impure molecules. The product was dried on the water bath and the product was obtained in the form of fine powder. Where the final yield obtained from 50g of *B. Orellana* seeds was 0.5g of the product Bixin and stored in 4˚ C.[12]Standardization of Bixin has been made by Ultra-violet spectroscopy, thin layer chromatography, Mass spectroscopy[13].

2.2.Ultra-violet spectroscopy: A stock solution of 10mg/ml of acetone of Bixin was made and then the four dilutions of the concentrations 2, 4, 6, 8 mg/ml respectively, was made by dissolving in acetone by withdrawing from a stock solution. And the absorbance of the sample was measuredat 200-600nm, at normal pressure and room temperature. Where the absorbance maxima of the standard were found to be 425 nm, 457nm, and 487 nm respectively (Abayomi *et al*., 2014).

1.3. Thin Layer Chromatography:

The stock solution of the Bixin was prepared in Ethanol 10mg/ml. The sample was prepared in ethanol and was loaded to silica plate, i n-butanol, methyl ethyl ketone and ammonia (3:2:2 by volume) and was employed as mobile phase. The solvent front was waited to be ascended for about 10cm.The sample mixture is allowed to dry. Where the standard RF values of Bixin appears to be 0.50. Then the sample solution is sprayed with 5% sodium nitrate and 0.5 mol/l sulphuric acid and the spots were made to decolourise[14][15].

1.4. Mass spectrometry

Sample for MS was prepared by tramp sampling along with headspace purge where it was directed in 10.0 ml of triplicate samples. The testers remained then eliminated into 100 mL of 30 ˚ C round-bottom flask for 30 minutes with nitrogen gas with the elimination flow of 880 ml/min in 26.4L onto a carbotop absorbent. Where Heptanol and ethyl-laureate is added in standard concentration of 0.5-1 µg/mL. Diethyl ether was used as desorbed analyte, with concentration of 75 µl and inoculated for Mass spectrum. 2 µl of sample inoculations were ended in the anchorage of injection in the injector in the split less mode. Column temperature was maintained up to 40˚ C for about 5 minutes. Mass-spectroscopy operating conditions was maintained up to 23˚ C, voltage ionization condition for about 70 eV and the scan range 33-300 at 2.76 scans[16][17,18]

1.5. Antioxidant Assay

DPPH Assay: DPPH is a radical which has long organic nitrogen radical stability with florid purple colour. It has proven for the action of stability and maximum absorbance of 517 nm. DPPH analyse is grounded on the basic principle of the DDPH is to reduce the colour yellow to the yellow diphenyl-picryl hydrazine. This can be assessed by Electron Spin Resonance or by the measure of decrease in absorbance. Stock solution of Ascorbic acid was prepared as 1 mg/ml in methanol. From the stock the concentrations were prepared as 2, 4, 6, 8 and 10 µg/ml respectively. Sample stock was prepared as 1 mg/ml in methanol, concentrations of 20, 40, 60, 80, and 100 µg/ml respectively was prepared. The solution of DPPH was prepared as 3.94 mg in 100 ml of methanol. 2 ml of 0.1mM of DPPH was added to 2 ml methanol, the absorbance was immediately measured at the wavelength of 517 nm, which served as control. 2 ml of DPPH was added to 2 ml of test extracts and shaken well. The test samples were incubated till 30 minutes. The absorbance was noted in the wavelength of 517 nm against methanol as blank. The whole experiment is carried out as triplicate. Then % scavenging activity and IC_{50} was calculated[19,20].

1.6. Induction of Depression in Mice

Mice were exposed to a sequence of minor stresses for 3 weeks to induce the various kinds of stressors like physical, behavioural, biochemical and physiological types. The treatment was initiated from the 1st day of induction parallelly along with the stress. Behavioural tests were conducted on the 0th day, 7th day, 14th day, 21st day. On the 22nd day, the animals were euthanized to collect the brain for further biochemical assays.[21]

1.7. Groups and treatment:

The dosage regimen was selected based on the antioxidant activity of the Bixin, to evaluate the antidepressant activity has been depicted in Table no. 2 (Silva *et al.*, 2001).

1.8. Behavioural assessments:

Animals were placed in the behavioural analysis room for about 1 hour before experimenting.[22]

1.8.1. Tail suspension test

The animals were adjourned to the air with the help of hook made of metal by the support of an adhesive tape enfolded around the tail, for about 15 cm above the ground level. The experiment noted for the initial dormancy to the complete interval of 6 minutes till the assessment period[23,24]

1.8.2. Open field test

Locomotory movement of the mice in Open field test was assessed by an opaque plastic box in the 30 X 30 X 30 cm dimensions. The peripheral group was subdivided into major sectors of centre and the central sector consists of 4 central squares. The mice were placed individually in the box with the fading light for about 10 minutes and the area was cleaned with 70% of the ethanol between each test.

2.8.2. Sucrose preference test

The animals were skilled for the recognition of the bottles while they were in the cages. The animals were subjected to the sucrose solution (1%). Where the animals were deprived of water for about 4 hours. The liquid consumption was calculated by the weight of the bottle[5].

$$Sucrose\ Peference = \frac{Sucrose\ Consumed}{Water\ Consumed + Sucrose\ Consumed} X100$$

2.9. Antioxidant Markers

Brain homogenate was prepared as per the standard procedure and the Total proteins [13] in the homogenate was estimated. Further antioxidant markers like Lipid Peroxidation **(**LPO)[13], Reduced glutathione(GSH)[25], Superoxide dismutase(SOD) were analysed.[26][27]

2.10. Acetylcholinesterase (AChE)assessment

AChE activity in the brain homogenate was determined using the colorimetric assay of Ellman et al. [16].

2.11. Histopathology

Briefly, brain samples (10% formalin) were kept in the fixative solution. The transverse section of the brain was made by using micro tomb device with a thickness of 4µm, staining was done by using haematoxylin and eosin solution and observed under (40X and 100X) magnification.

2.12. Data analysis

The behavioural analysis was examined by one Way ANOVA tailed by post-hoc Bonferroni test. Where the Antioxidant markers were analysed by One Way ANOVA with post-hoc Tukey test. All the statistics were expressed in mean ± SEM.

3. Results and Discussion

3.1. Qualitative analysis of Bixin

3.1.1. Ultraviolet Spectroscopy of Bixin

Various concentrations of the Bixin were tested for the absorbance maxima in the ultraviolet spectroscopy the standard absorbance maxima appears to be 458 nm and the various concentrations of Bixin appears to be 458nm, 487nm, 458nm, & 458 nm respectively. And the standard and test Figures has been shown in the figure no. 1 & 2 respectively along with the R square value in figure no. 3

3.1.2. Thin layer chromatography of Bixin

The Rf value of the standard Bixin taken from stock 10 mg/ml is termed to be 0.6 and the rf value of mg/ml, the extra

3.1.3. Mass spectroscopy of Bixin

The mass spectroscopy of the Bixin was run where the standard Bixin isomer 9'-Z has shown the intensity of $[M+H]^{+}$ of 393, $[M+H-H20]^{+}$ of 377, and [M+H-HOCH3] of 363 and the sample Bixin ran was found to be the intensity of 393.1450, 395.175, 349.1687 respectively where the various chemical shifts in the conjugation of the theme has not been assigned The standard Bixin has been depicted in the figure no. 5 and the test Bixin has been depicted in figure no. 6

3.2. *In-vitro* antioxidant assay

3.2.1. DPPH Assay: Free radicle scavenging activity of the Bixin by DPPH method is shown in the below Table .5.2. The Bixin has moderately scavenged DPPH radical with the IC_{50} of 72.21± 0.56 and the standard compound was taken to be ascorbic acid has shown the IC_{50} of 4.95±0.008 it has been tabulated in Table no. 3.1

3.3. Tail Suspension Test

Tail suspension is of the main stressors used for the evaluation of the depressive-like behaviour in the mice. Three weeks of chronic unpredictable mild stress has significantly (p< 0.0001) elevated the immobility in the control group on 0^{th} day has shown the results from (103.67± 0.71 to 156.83±1.71) as compared with the normal group from (107.33 ± 0.81 to 86.50±2.13) Bixin 50 mg/kg has shown the decrease in the immobility from (104.17± 1.31 to 77.33 ± 0.91) and Bixin 100 mg/kg has shown the significant (p< 0.0001) decrease from (105±0.72 to 80.33±1.21) as that of standard Fluoxetine 20mg group from (106.50±1.14 to 81.50±0.61)showed in Figure no.3.7.

3.4. Sucrose PreferenceTest

The sucrose preference test is used to evaluate the % of anhedonia by sucrose, by evaluating the palatable sucrose consumed by the mice, In the recurrent administration of Chronic Unpredictable Mild Stress for three weeks the percentage of sucrose consumed has significantly (p< 0.05) decreased in the control group from (60±0.02 to 37±0.04) when compared with the normal group(50±0.02 to 40±0.01), where Bixin 50 mg/kg and Bixin 100 mg/kg tend to show the noteworthy (p < 0.05) consumption of sucrose as (50±1.56 to 50 ±1.25) and (60.15±60±0.96) respectively to as that of group Fluoxetine 20 mg/kg (50±1.24 to 50 ± 0.94) in 21 days interval of time where, has been shown in the figure no.3.8.

3.5. Open field test

The probable changes in the HPA axis will show the variation in the movements. The assessment of Chronic Unpredictable Mild Stress was done in three sets in the Open Field Test, viz; Number of the line crossed, freezing time, and Time spent in the corner. The readings were shown in number of line crossed was significantly decreased (p< 0.0001) in the

control group (83.00±1.65 to 54.89±2.75) as compared with the normal. Where Bixin 50 mg/kg and Bixin 100mg/kg has shown the significant ($p < 0.0001$) stability as (77.51±0.61 and 71.64±1.51) and (76.23±1.91 to 75.69 ±5.63) with that of Number of lines crossed as that of Standard fluoxetine 20 mg/kg (76.56±1.45 to 80.89±3.63). Where the Freezing time in the control group has shown significant increase ($p< 0.0001$) in the time frozen as (25.56±2.32 to 44.32±0.98) compared to that of normal group. Where the Bixin 50 mg/kg and Bixin 100 mg/kg has shown the significant improvement ($p < 0.0001$) as (23.32±2.15 to 27.51±1.71) and (21.61±1.65 to 26.84±1.55) that of Fluoxetine 20 mg/kg (21.68 ±1.69 to24.56 ± 2.25). The time spent in the corner was significantly ($p< 0.0001$) more in the control group as (20.35±4.17 to 95.32±2.25) when compared with normal group. Where the time spent corner was significantly decreased ($p< 0.0001$) in the group of Bixin 50 mg/kg and Bixin 100 mg/ kg as (23.35± 2.10 to 38.31± 6.01) and (23.39± 4.22 to 30.89 ± 0.15) that of Standard fluoxetine 20 mg/kg as(25.00±2.34 to 30.00 ±1.25)has been shown in the Figure no. 3.11.

3.6. Antioxidant markers of CUMS

3.6.1. Total protein

Administration of Bixin has shown the significant ($p< 0.05$) activity in the protein control group (0.74± 0.09) as compared with the normal group (0.37±0.02)However the activity of Bixin 50 mg/kg and Bixin 100 mg/kg is noteworthy(0.81±0.07and 0.86±0.07) respectively same as that of Standard Fluoxetine 20 mg/kg (0.63± 0.05)has been depicted in the Figure no.12

3.6.2. Superoxide dismutase (SOD)

SOD serves a key role in the antioxidant property. SOD is an important enzyme family in living cells for maintaining normal physiological conditions and for coping the stress. Induction of Chronic Unpredictable Mild Stress for 3 weeks has significantly ($p< 0.05$) decreased SOD activity in the control group (52.82±7.75) as compared to the normal group (75.80±2.84). Bixin 50 mg/kg and Bixin 100 mg/kg has shown activity as (71.91 ±2.41 and 76.42±4.62) respectively that of Fluoxetine 20 mg/kg, standard group (71.52±5.12) has been depicted in Figure no. 3.13

3.6.3. Lipid Peroxidation (LPO)

The central nervous is vulnerable to LPO owing to high brain oxygen consumption and also to its rich polyunsaturated content. It is relatively deficient in antioxidant enzymes as well. A significant increase in the malondialdehyde concentration in the brain has shown significant ($p < 0.05$) raise in the control group (16.62±1.41) when compared with the normal group (10.96±0.97). The Bixin 50 mg/kg and Bixin 100 mg/kg has significantly ($p < 0.05$) decreased (7.61±2.71 and 7.91±2.31) in the malondialdehyde concentration as that the standard (8.91±2.71) has been shown in Figure no. 3.14

3.6.4. Reduced glutathione (GSH)

Glutathione plays an important role in the detoxification of reactive oxygen species in the brain, the alteration in enzyme activity or the concentration of small molecular weight, leads to depressive disorder. The Chronic Unpredictable Mild Stress has significantly ($p< 0.05$) reduced the glutathione in the brain in the control group (4.86±0.29) as compared to that of the normal group (2.52±0.21). Bixin 50 mg/kg and Bixin 100 mg/kg has shown the significant ($p< 0.05$) increase in the brain (3.61±0.43 and 3.83±0.39) as that of Standard compound Fluoxetine 20 mg/kg (4.89±0.29) has been shown in Figure no. 3.15

3.7. Acetylcholine Esterase activity in Brain

AChE is the key enzyme in the cholinergic nervous system, during the progression of depression, many different types of neurons disorient and there will be profound loss in the prefrontal cortex, with decline in ACH. The administration of the Chronic Unpredictable mild stress has created a significant ($p > 0.05$) increase AChE activity in the control group (71.92±9.22) as compared with the normal (62.07±5.81)Where the Bixin 50 mg/kg and Bixin 100 mg/kg has decreased in the AChE activity (63.31±3.41 and 63.51±5.36) respectively as that of the standard Fluoxetine 20 mg/kg (60.70±7.82) has been shown in Figure no. 3.16.

Table 2.1. A protocol of Chronic Unpredictable Mild Stress for 3 weeks

Stress	Narration	Duration
Food deprivation	Food was withdrawn	Overnight
Water deprivation	Water was withdrawn	Overnight
Novel odour	Novel odour was given for animals	1 hour
Novel objects	Novel object was placed in the cage	5 hours
Soiled bed exposure	Rat scope of bedding was placed in the cages of the mice	2 hours
Cage tilting	Cages was titled to the back (45 degrees)	2 hours
Tail clipping	Mouse tail was clipped	5 minutes
Restraint stress	The mouse was placed in a restrainer (falcon tube)	30 minutes
Wet sawdust	The husk was wetted with water	2 hours

Table no. 2.2 Groupings of animals and dosage schedule

S.no	Group (n=6)	Treatment	Dose (p.o)
1	Normal	Unstressed + CMC	1% of CMC, 10 ml/kg; Once daily
2	Control	Stressed+ CMC	1% of CMC, 10 ml/kg; Once daily
3	Standard	Stressed+ Fluoxetine	20 mg/kg; Once daily
4	Test 1	Stressed+ Bixin (low dose)	50 mg/kg; Once daily
5	Test 2	Stressed +Bixin (high dose)	100 mg/kg; Once daily

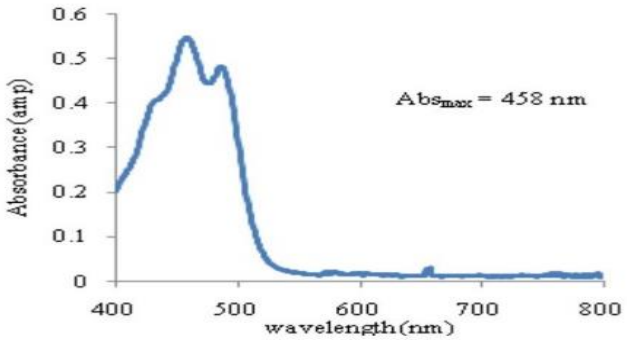


Figure 3.1. Standard absorbance maxima of bixin

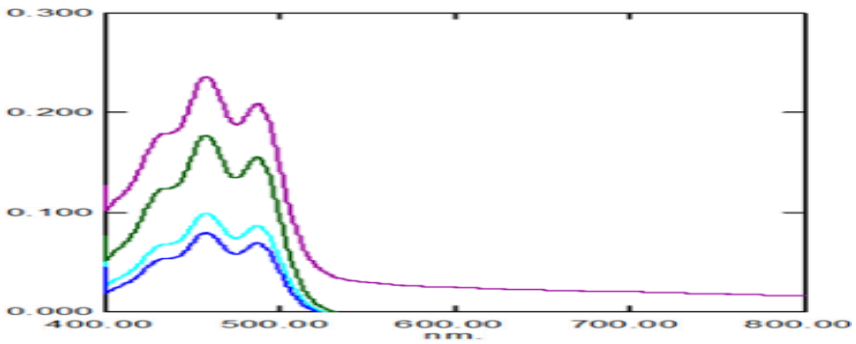


Figure 3.2. Absorbance maxima of Extracted Bixin

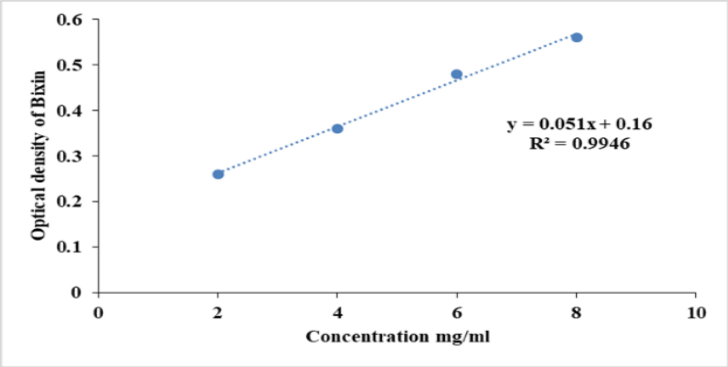
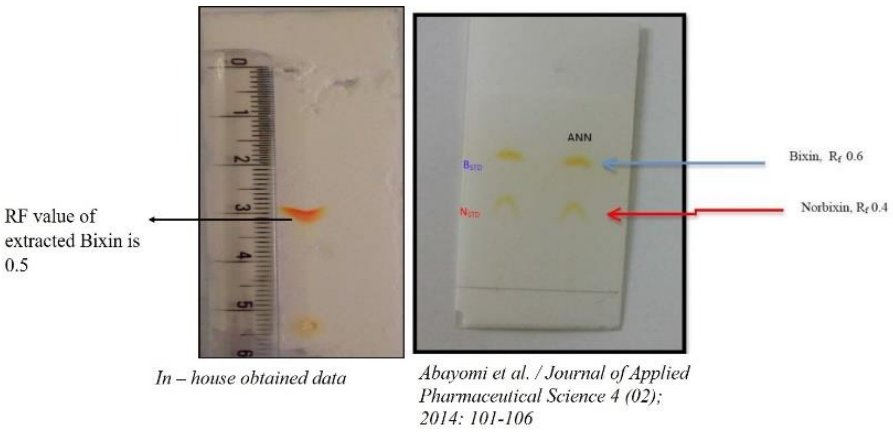


Figure 3.3 Optical density of Bixin



In – house obtained data

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Figure 3.4. (a) Rf value of extracted bixin and (b) Rf value of standard bixin

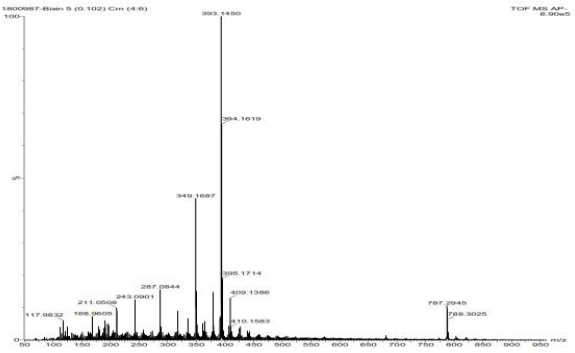

Figure 3.5. Mass spectroscopy of standard bixin

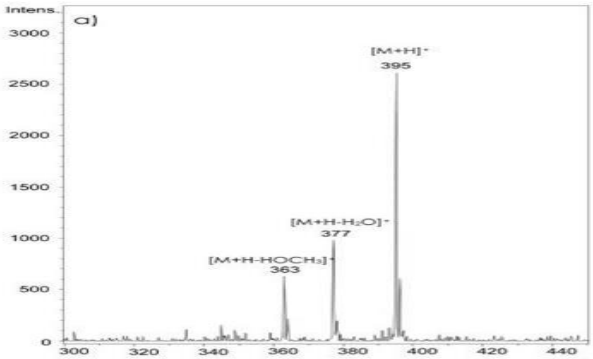

Figure 3.6.Mass spectroscopy of extracted Bixin

Percentagescavenging activity			
Conc (mg/ml)	**Ascorbic acid**	**Conc (mg/ml)**	**Bixin**
2	34.867±5.276	20	25.14 ± 0.400
4	41.282±4.702	40	30.66±0.734
6	58.061±4.031	60	44.49±2.795
8	66.546±3.126	80	54.32±2.998
10	77.362±1.629	100	64.24±4.938
IC $_{50}$	**4.95 ± 0.008**	**IC $_{50}$**	**72.21 0.56**

Table no. 3.1. Percentagescavenging activity of Ascorbic acid

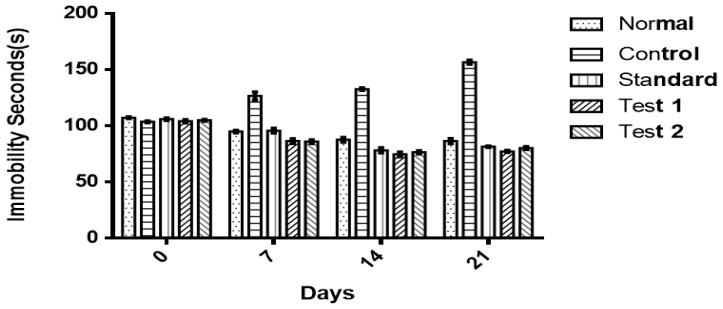

Figure no. 3.7. shows about the Effect of Bixin on Immobility by CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *two-way ANOVA* followed by Bonferroni's post-t-test**.** $^{a}p < 0.0001$ Vs normal group.$^{b}p < 0.0001$ vs control group.

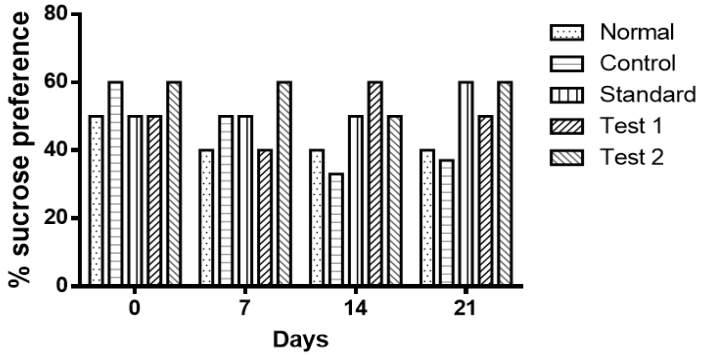

Figure no.3.8. shows about the Effect of Bixin on Sucrose preference test on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *two-way ANOVA* followed by Bonferroni's post-t-test**.** $^{a}p < 0.05$ Vs normal group.$^{b}p < 0.05$ Vs control group

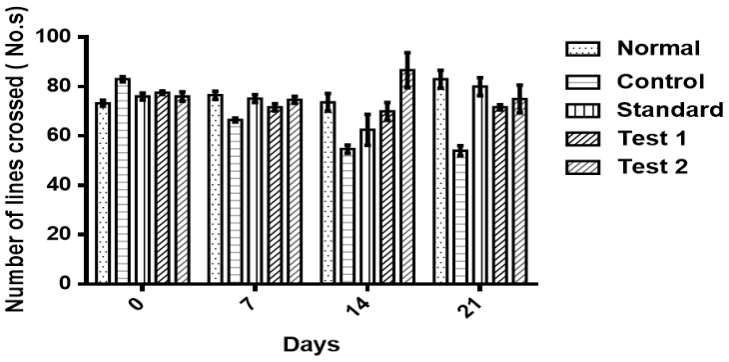

Figure no. 3.9. shows about the Effect of Bixin on a number of lines crossed in Open field test on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *two-way ANOVA* followed by Bonferroni's post-t-test**.** [a]*p*< 0.0001 Vs normal group.[b]*p*< 0.0001 vs control group.

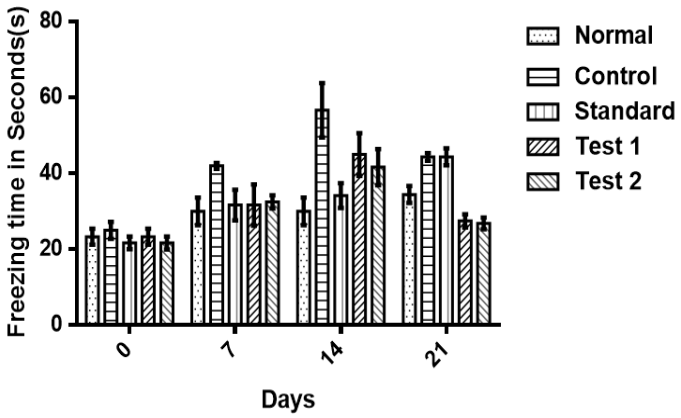

Figure no 3.10. Shows about the Effect of Bixin on freezing time Open field test on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *two-way ANOVA* followed by Bonferroni's post-t-test**.** [a]*p*< 0.0001 Vs normal group.[b]*p*< 0.0001 vs control group.

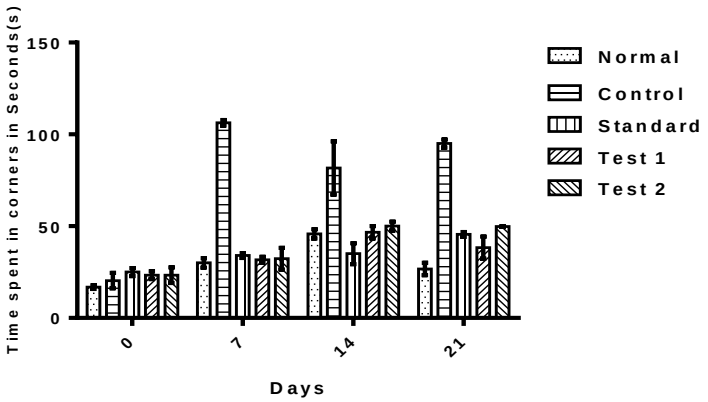

Figure no.3.11. shows about the Effect of Bixin on time spent in coroners Open field test on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *two-way ANOVA* followed by Bonferroni's post-t-test**.** [a]*p*< 0.001 Vs normal group.[b]*p*< 0.001 Vs control group.

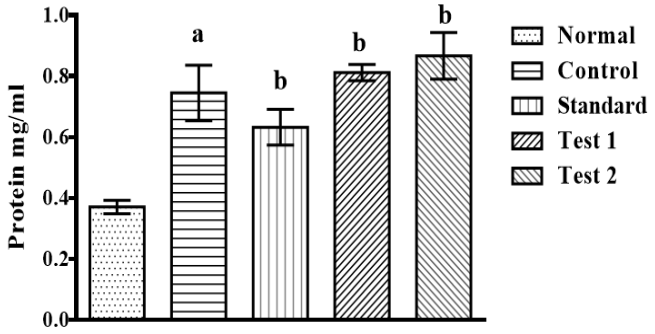

Figure no.3.12. Shows the Effect of Bixin on protein in the brain on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *one-way ANOVA* followed by Tukey test**.** [a]*p*< 0.05 Vs normal group.[b]*p*< 0.05 Vs control group.

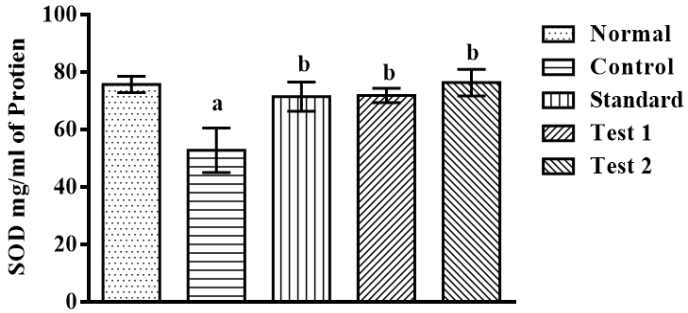

Figure no.3.13. shows the Effect of Bixin on Superoxide dismutase in the brain on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by one-way *ANOVA* followed by Tukey test**.** [a]*p*< 0.05 Vs normal group.[b]*p*< 0.05 Vs control group.

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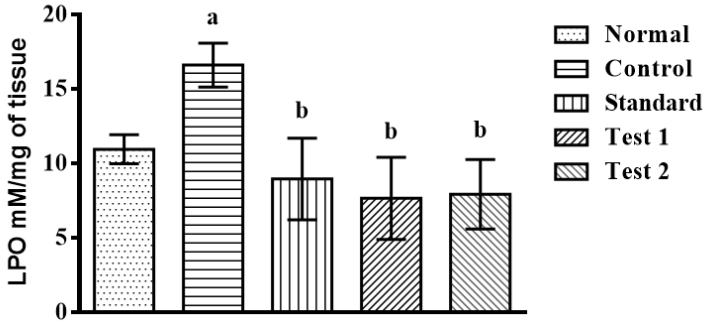

Figure no.3.14. shows about the Effect of Bixin on Lipid peroxidation in the brain on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *one-way ANOVA* followed by Tukey test**.** [a]*p*< 0.05 Vs normal group.[b]*p*< 0.05 Vs control group.

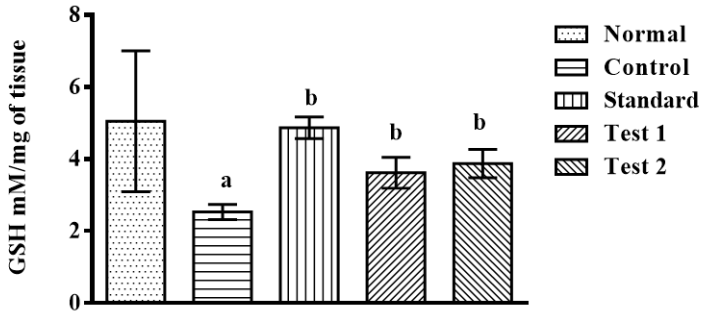

Figure no.3.15. shows the Effect of Bixin on Reduced Glutathione in the brain on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *one-way ANOVA* followed by Tukey test**.** [a]*p*< 0.05 Vs normal group.[b]*p*< 0.05 Vs control group.

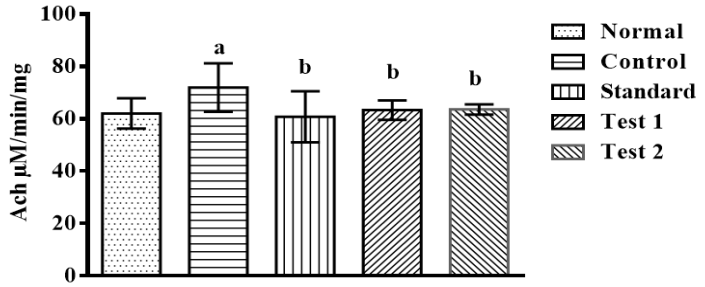

Figure no.3.16. shows the Effect of Bixin on Acetylcholine Esterase in the brain on CUMS. Data were expressed as mean ± SEM, n=6. Data were analysed by *one-way ANOVA* followed by Tukey test**.** [a]*p* < 0.05 Vs normal group.[b]*p*< 0.05 Vs control group.

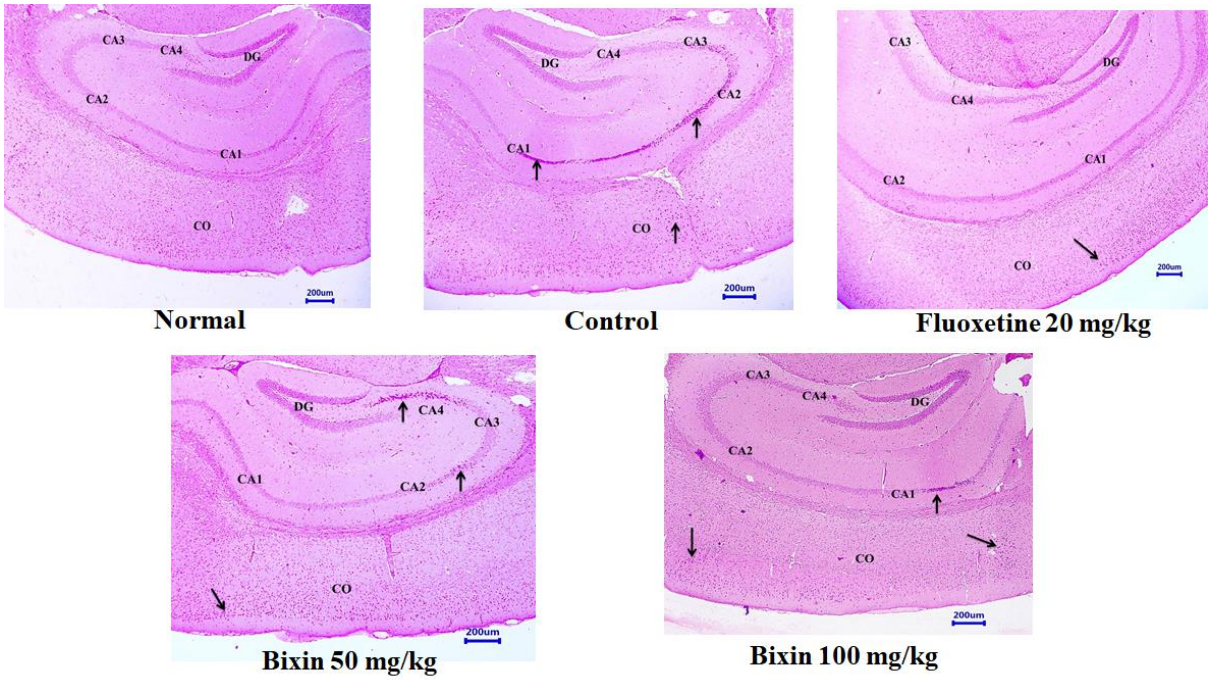

Figure no.3.17. Effects of Bixin on Chronic Unpredictable Mild stress-induced depression on the various treatment of Vehicle, Standard and test drugs in the hippocampus of brain samples.

3.8. Histopathology of CUMS

The Chronic Unpredictable mild stress model has been evaluated for the Histopathological studies. Normal group histology of hippocampus CA1, CA2, CA3, CA4 regions and dentate gyrus (DG) and cortex regions have not changed. The histology of control group has shown neuronal degeneration at the cortex and reduced neuronal cells CA1 and CA2 regions of the hippocampus shown in Figure no. 3.17.

CONCLUSION

The present study is to comprehend about the antioxidant property of Bixin can help in the depression caused by Chronic Unpredictable Mild Stress-induced depression a newer approach in the treatment of depression with the food colorant that has abundant antioxidant property. A decrease in the SOD and increase in the LPO lead to an imbalance in the antioxidant defence mechanism that has been induced with CUMS, GSH helps in removing ROS from the brain metabolite but, it was also significantly decreased due to CUMS. However, Bixin helped to maintain homeostasis of the antioxidant enzymes and also helped in elevating GSH levels responsible for depleting ROS from the brain. A decreased AChE activity along with the improvement in behavioural abilities after treatment with Bixin leads us to believe that it also helps in cognitive enhancement during depression.In the present study, the induction of Chronic Unpredictable Mild Stress for 3 weeks has caused severe depression and anxiogenic nature in mice. Where depression was ameliorated by Bixin, as a result of the improvement in the Behavioural, Biochemical and Histological alterations.

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Abbreviations: NWM-Nicotine Withdrawal Model, LPO- Lipid Peroxidation, SOD-Superoxide dismutase.

REFERENCES:

1. Gabrilovich E. Computing Semantic Relatedness Using Wikipedia-based Explicit Semantic Analysis. :6.
2. Dhingra D, Valecha R. Punarnavine, an alkaloidisolated from ethanolic extract of BoerhaaviadiffusaLinn.reverses depression-like behaviour in mice subjected to chronic unpredictable mild stress. IJEB Vol52(08) [August 2014] [Internet]. 2014 Aug [cited 2020 Apr 7]; Available from: http://nopr.niscair.res.in/handle/123456789/29298
3. Murad F, Atta-ur-Rahman, Bian K. Cancer Therapy. Bentham Science Publishers; 2019. 302 p.
4. Black CN, Bot M, Scheffer PG, Cuijpers P, Penninx BWJH. Is depression associated with increased oxidative stress? A systematic review and meta-analysis. Psychoneuroendocrinology. 2015 Jan;51:164–75.
5. Animal models as simulations of depression. Trends in Pharmacological Sciences. 1991 Jan 1;12:131–6.
6. Feng M, Crowley NA, Patel A, Guo Y, Bugni SE, Luscher B. Reversal of a Treatment-Resistant, Depression-Related Brain State with the Kv7 Channel Opener Retigabine. Neuroscience. 2019;406:109–125.
7. Models of Depression: Unpredictable Chronic Mild Stress in Mice - Nollet - 2013 - Current Protocols in Pharmacology - Wiley Online Library [Internet]. [cited 2020 Apr 7]. Available from: https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/0471141755.ph0565s61

8. Laurenti D. Identification of novel neuroprotective genes against oxidative stress : a combined genetic and proteomic approach. 2015 [cited 2020 Apr 7]; Available from: https://researchdirect.westernsydney.edu.au/islandora/object/uws%3A36210/
9. Biosynthesis of the Food and Cosmetic Plant Pigment Bixin (Annatto) | Science [Internet]. [cited 2020 Apr 7]. Available from: https://science.sciencemag.org/content/300/5628/2089
10. Kakkar V, Singh S, Singla D, Kaur IP. Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin. Molecular Nutrition & Food Research. 2011;55(3):495–503.
11. Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal. 2008;54(4):733–749.
12. Preston HD, Rickard MD. Extraction and chemistry of annatto. Food Chemistry. 1980 Jan 1;5(1):47–56.
13. Dendy DAY. **Annatto, the Pigment of***Bixa Orellana*. East African Agricultural and Forestry Journal. 1966 Oct;32(2):126–32.
14. Journal of Applied Pharmaceutical Science [Internet]. [cited 2020 Apr 7]. Available from: https://www.japsonline.com/abstract.php?article_id=1193
15. Mínguez-Mosquera MI, Hornero-Méndez D, Garrido-Fernández J. Detection of bixin, lycopene, canthaxanthin, and beta-apo-8'-carotenal in products derived from red pepper. J AOAC Int. 1995 Apr;78(2):491–6.
16. Characterization of bixin by LC-MS and LC-NMR - Rehbein - 2007 - Journal of Separation Science - Wiley Online Library [Internet]. [cited 2020 Apr 7]. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jssc.200700089
17. Holtin K, Kuehnle M, Rehbein J, Schuler P, Nicholson G, Albert K. Determination of astaxanthin and astaxanthin esters in the microalgae Haematococcuspluvialis by LC-(APCI) MS and characterization of predominant carotenoid isomers by NMR spectroscopy. Analytical and bio analytical chemistry. 2009; 395(6):1613.
18. Wolfender J-L, Marti G, Ferreira Queiroz E. Advances in techniques for profiling crude extracts and for the rapid identificationof natural products: Dereplication, quality control and metabolomics. Current organic chemistry. 2010;14(16):1808–1832.
19. Kinattingal N, Mruthunjaya K, Mahalakshmi A, Manjula S. Cardioprotective effect of Tamarindusindicalinn against isoproterenol induced myocardial infarction in rats. Int J Pharm Pharm Sci. 2016;8:254–60.
20. Quintero Quiroz J, Naranjo Duran AM, Silva Garcia M, Ciro Gomez GL, Rojas Camargo JJ. Ultrasound-Assisted Extraction of Bioactive Compounds from Annatto Seeds, Evaluation of Their Antimicrobial and Antioxidant Activity, and Identification of Main Compounds by LC/ESI-MS Analysis. Int J Food Sci. 2019; 2019:3721828.
21. Induction of depression-related behaviors by reactivation of chronic Toxoplasma gondii infection in mice. - PubMed - NCBI [Internet]. [cited 2020 Apr 16]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26554725
22. Olney JJ, Marshall SA, Thiele TE. Assessment of depression-like behavior and anhedonia after repeated cycles of binge-like ethanol drinking in male C57BL/6J mice. PharmacolBiochemBehav. 2018;168:1–7.
23. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. NeurosciBiobehav Rev. 2005;29(4–5):571–625.
24. Poleszak E, Szopa A, Bogatko K, Wyska E, Wośko S, Świąder K, et al. Antidepressant-Like Activity of Typical Antidepressant Drugs in the

Forced Swim Test and Tail Suspension Test in Mice Is Augmented by DMPX, an Adenosine A2A Receptor Antagonist. Neurotox Res. 2019 Feb;35(2):344–52.

25. Prabhakar PV, Reddy UA, Singh SP, Balasubramanyam A, Rahman MF, Indu Kumari S, et al. Oxidative stress induced by aluminum oxide nanomaterials after acute oral treatment in Wistar rats. Journal of Applied Toxicology. 2012;32(6):436–445.
26. Wills ED. Lipid peroxide formation in microsomes. Relationship of hydroxylation to lipid peroxide formation. Biochemical journal. 1969;113(2):333–341.
27. Vaváková M, Ďuračková Z, Trebatická J. Markers of Oxidative Stress and Neuroprogression in Depression Disorder. Oxid Med Cell Longev. 2015;2015:898393.