# BIOCHEMICAL EVIDENCES OF ARGEMONE MEXICANA ON STRESS AND NOOTROPIC ACTIVITY

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**Abstract**

The present study was designed to evaluate the effect of Mexican poppy on memory of unstressed and stressed Swiss young albino mice. Mexican poppy (500mg/kg i.p) and piracetam (200 mg/kg i.p.), were administered for 21 successive days to separate groups of unstressed and stressed mice. The nootropic activity was evaluated using elevated plus maze and Hebbs Williams Maze. Brain acetylcholinesterase (AChE), brain nitrite and plasma corticosterone levels were also estimated. unpredictable chronic mild stress was produced by using different stressors. Mexican poppy (500mg/kg i.p) and piracetam (200 mg/kg i.p.), significantly showed memory enhancing activity in both unstressed and stressed mice. Mexican poppy significantly reduced brain AChE activity and brain nitrite levels in both unstressed and stressed mice. Mexican poppy (500mg/kg i.p) significantly reversed scopolamine-induced amnesia in unstressed and stressed mice. 7-Nitroindazole [a neuronal nitric oxide synthase (NOS) inhibitor] and aminoguanidine (an inducible NOS inhibitor) significantly enhanced memory improving activity and brain nitrite decreasing effect of Mexican poppy in unstressed and stressed mice respectively. Plasma corticosterone levels were significantly decreased by Mexican poppy (500mg/kg i.p) in stressed mice as compared to its control. Thus, Mexican poppy showed memory enhancing activity in unstressed mice probably by decreasing brain AChE activity and by inhibition of neuronal NOS. The memory enhancing activity of Mexican poppy instressed mice might be due to decrease in brain AChE activity, inhibition of inducible NOS and by decreasing the elevated plasma corticosterone levels.

**Keywords:** Acetylcholinesterase UCMS Naringin Inducible NOS Memory Neuronal NOS

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**Abbreviations**:-
AD-Alzheimer Disease
AchE- Acetylcholinesterase
NO- Nitric oxide
NOS- Nitric oxide synthase
iNOS- Inducible nitric oxide synthase
nNOS- Neuronal nitric oxide synthase
EPM- Elevated plus maze
HWM- Hebbswilliam maze
TRC- Time to reach reward chamber
TL- Transfer Latency
7-NI- 7-Nitroindazole
AG- Aminoguanidine
UCMS- Unpredictable chronic mile stress
HIV- Human immunodeficiency virus

## 1. INTRODUCTION

Learning implies osmosis of grouped abilities and information, while memory identifies with how the brain stores and reviews data. It's practically unimaginable for an individual to truly get the hang of something without additionally having the memory to hold what they need realizing. Here and there, learning and memory keep up an extremely reliant relationship, one that is undeniably more nuanced and convoluted than it will seem to jump on a superficial level. Learning and memory are close with respect to each other. Learning is that the obtaining of aptitude or information which needs adaptability, while memory is that the statement of what you've gained which needs strength [1]. The idea and meaning of nootropicwas first proposed 1n 1972 by Guirgea who begat the term ‘nootropic’ from Greek words ‘noor’ (mind) and ‘tropin’ (to turn towards) to mean improvement in learning and memory. Another distinction is that the speed with which learning of the most recent aptitudes and information might be a moderate procedure while if the obtaining happens in a flash that is making a memory. As per the WHO, Trusted Source says that 47.5 million individuals inside the world live with dementia. Dementia is a very disorder that applied to a lot of manifestations that adversely sway intellectual capacities like memory and thinking, yet Alzheimer's is a dynamic neurodegenerative ailment that causes weakness in memory and psychological capacity. Dementia is an umbrella that covers Alzheimer's illness. Dementia is a general term to depict side effects influence memory and day by day exercises, Alzheimer's ailment is that the commonest sort of dementia [2]. Stress is known to prompt alteration in different physiological reactions and influence the physiological balance which results in deterioration of homeostasis. Every human being today faces unpleasant circumstances in everyday life and overemphasize has been proposed to be engaged with pathogenesis of different disease states like nervousness, diabetes mellitus, peptic ulcer, sexual dysfunctions, cognitive impairment, hypertension. The Stress reactions are sub served by an unpredictable framework with the resulting activating hypothalamic pituitary adrenal axis. Stressor acts on both physically and mentally on neuronal pathway and lead to an increment in emission of three hormones corticotrophin releasing hormone (CRH), adrenal corticotrophin hormone (ACTH) and cortisone. These three hormones have a significant effect on metabolic capacities. Increase in the level of cortisol directly increases the blood glucose level, and cholesterol levels [3].In excess of hundred synapses engaged with keeping up the subjective capaies like acetylcholine, epinephrine and norepinephrine, GABA, dopamine, serotonin, neuropeptides, angiotensin changing over proteins, nitric oxide etc. Acetylcholine - a crucial synapse for intellectual capacities. At the point when the degree of acetylcholinesterase (AChE) is expanded it brings about the inadequacy of acetylcholinefunctionswhich leads tocognitive impairment inside the person. Therefore, the target of cholinergic system patways might be among the objectives for the cure of cognitive disability [4]. Stress likewise assumes a pivotal job in neurodegeneration. Stress is a significant explanation behind the arrival of corticosterone in blood. Stress-instigated increment in corticosterone levels is the main factor behinddebilitates memory capacities. Nitric-oxide (NO) is created l-arginine in the cerebrum. Chronic Stress in all animals fundamentally elevates brain nitrite. 7-Nitro-indazole and aminoguanidin are selective inhibitors of neuronal NOS and inducible - NOS which help in suppression of nitric oxide synthase, demonstrated nootropic activity in animals [5].

Mexican poppy might be a thorny, bald, expanding herb with yellow juice and conspicuous yellow blossoms. The biological name of Mexicana poppy is ‘Argemone mexicana’. The plant is pantropic in dispersion and it's a weed in squander places. It's local to America and naturalized all through India. The regular Indian name of Mexican poppy is Satyanashi with Papaveraceae family. It's likewise referenced as "kateli ka Phool" in India. Mexican poppy is comprehensively utilized as conventional dosing for the healing of different infections. Different pieces of the plant were broadly used in A.Y.U.S.H drugs. This plant shows different movement like antimicrobial action, wound mending capacity, anti-larval and chemo-sterilant action, anti-nematode, anti-malarial, anti-bacterial [6] and fungicidal, anti-cancer [7], liver-protective [8], hostile to H.I.V, anticonvulsant [9] and neuro-pharmacological action [10]. Phytochemical

screening of this plant has uncovered the nearness of alkaloids, flavonoids, amino acids, and unsaturated fats. Mexican poppy has indicated a guarantee as a proficient biocontrol operator. In any case, there are no precise pharmacological examinations to help the nootropic action of Mexican poppy. Thus, this examination has been expected to investigate the impact of Mexican poppy on the cognition of non-stressed and stressed S.A male mice further, we additionally considered the conceivable association of nNOS and iNOS; and furthermore, evaluated nitrite level, AChE level in brain andcorticosterone level in plasma of mice. Mexican poppy (500 mg/kg) was chosen by prior examinations where Mexican poppy have been accounted for to dynamic in neuro-pharmacological studies [10]

## 2. Material method

### 2.1 The assortment of plant extricate

The entire Argemone mexicana plant was gathered in June 2018 from the Jhajjar region, Haryana (India). The plant example was confirmed from the CSIR-National organization of science correspondence and data assets Delhi with ref. no-NISCAIR/ RHMD/ Consult/2018/3284-85. Plant material was dried and powdered for extraction. The coarsely powdered plant (600g) of Mexicana poppy was independently exposed to extraction utilizing alcoholic concentrate for 10 days by cold maceration. The alcoholic extract was concentrated by rotating the vacuum evaporator under decreased pressure and afterward dried in outside.

### 2.1.1Alcoholic concentrates

In a firmly fixed holder innormaltemperature, 250gm of grounded plant material was extricated with 650 mL liquor. The concentrate was shielded from direct light and kept for the time being on a rotary shaker. The concentrate was sifted with a five-sheet of sterile muslin material. This technique was rehashed multiple times to get apparent and dismal filtrate. The liquor from the filtrate was evacuated by revolving dissipation. Concentrates were put away at very cold temp.(−16 ◦C)for the night and were accordingly freeze-dried at −60 ◦C in a 20 mL vacuum for 24 hrs. The concentrate was then cleaned with UV light and put away in a sealed shut compartment at 4 ◦C for additional utilization [10]

### 2.2. Test Animals

Swiss male albino mice, weigh around 30-35 gm were utilized for the reason. Mice were put independently in gatherings of 6 for every confine under research facility conditions with rotating light and a dull pattern of 12 hrs each. Nourishment and water were accessible to the mice. Mice were kept in the trial space for accommodation, at any rate, ten days before conduct tests. All the investigations were completed somewhere in the range of 09:00hrs-17:00 hrs. The test convention was endorsed by the Institutional Animals Ethics Committee (IAEC) of SGT University Gurugram, Haryana with ref.no-SGTU/IAEC/2018/06.

### 2.3. Drugs and Chemicals

L-arginine and acetylcholine iodide, were purchased from (Hi-Media lab Pvt. Ltd., Mumbai, India); piracetamwas purchased from (Ranbaxy New Delhi, India); Aminoguanidine, 7-Nitroindazole, Metyrapone, scopolamine were purchased from (Sigma-Aldrich, USA); were used in the present study. And other chemical were used of analytical grade.

### 3.Unpredictable Chronic Stress in mice

The mice wereput under stress as according tothe procedure of kumar et.al[11] [5]. Animals wereput under stress proceduresingle time per day for three weeks between 09:00hrs-14:00 hrs.

The sequence of stress is as under:

| Weeks | Mon. | Tues. | Wed. | Thu. | Fri. | Sat. | Sun. |
|---|---|---|---|---|---|---|---|
| Week 1 | C-Cold swiming(12°C,5 min) | T-tail pinch (30 s) | F-food and water removal (24 h) | O-overnight enlightenment | N-none | S-swimming at room temperature (23±2°C, 15 min) | T1-tail pinch (60 s) |
| Week 2 | C - Cold swiming(12°C,5 min) | O - overnight enlightenment | N -none | S1 swimming at room temperature (23±2°C, 10 min) | T2 $T_2$—tail pinch (90 s | C - Cold swiming(12°C,5 min) | F- food and water removal (24 h) |
| Week 3 | O - overnight enlightenment | N -none | T1- tail pinch (60 s) | S- swimming at room temperature (23±2°C, 15 min) | C- Cold swimming (12°C,5 min) | O - overnight enlightenment | F- food and water removal (24 h) |

## 4. Extereoceptive behavioral models for mice

### 4.1. Hebb Williams Maze

HWM is a reward based model valuable for estimating the spatial working memory of rodents. Quickly, HWM for mice is square box with length and width (60x60cm) having three division with start box then middle area and on opposite end a goal box which contain a reward in the form of food. Each of the three segments is removable entryways with cardboard. Before testing, animal were food restricted so that they are motivated to get food. Scheduled the primary day (i.e., ninth day of drug

dosing), put the mice in maze chamber keeping the entryway opened to encourage the passage of mice into the following compartment. The entryway of the beginning compartment is shut following the mouse move into the following compartment to forestall back passage. Time which is taken by the mouse to arrive at the goal compartment (TRC) from the beginning compartment on first day mirrors the learning list. Every mouse is permitted to investigate the labyrinth for 2minute with all the entryways opened before coming back to start box. Memory score of this educated undertaking will be analyzed 24 hrs after the primary day of preliminary. A huge decrease in TRC esteem shows a progress of memory [12].

Mice were separated into 10 groups and each group has minimum 6 animal, different animals were used for each experiment

Group I, II, and III: Normal saline (control), an alcoholic concentrate of Mexican poppy (500mg/kg i.p) and standard drug piracetam (200 mg/kg i.p.), individually be given for twenty days progressively. After given the drugs dosing TRC was recorded after 35 minutes on the twentieth day (learning). Memory was analyzed 35 minutes after drugs dosing on the last day.

Group IV and V: Normal saline and alcoholic concentrate of Mexican poppy (500mg/kg i.p) independently be infused for twenty days progressively. After the infusion of drugs on the twentieth day TRC was recorded after 35 min. Scopolamine (0.4 mg/kg) was infused after 24 hrs of these drugs, and memory was inspected 35 min after scopolamine infusion.

Groups VI, VII and VIII: Normal saline, an alcoholic concentrate of Mexican poppy (500mg/kg i.p) and piracetam (200 mg/kg i.p.), separately were managed for 20 progressive days alongside the stress strategies. On twentieth day after 35 minutes of drugs dosing TRC was recorded. After 24 hrs, TRC was recorded 35 min of drugs dosing.

Groups IX and X: alcoholic concentrate of Mexican poppy (500mg/kg i.p) and Normal saline individually were given for 20 successive days. On the last day TRC was noted after 35 min of drugs dosing. After 24 hrs, after given of all drugs after 35 minutes amino guanidine (50 mg/kg, i.p.) was infused and TRC was noted 10 min in the wake of liberating the mouse

Same groups were used for elevated plus maze to check the transfer latencies after 20 days washout period

**4.2. Elevated plus-maze Plus Maze**

Elevated plus-maze in addition to the labyrinth is an impartial behavioral model generally used to check the impact of different drugs dosing on memory. The strategy, system, along with endpoint for testing learning and memory are the same according to the parameters depicted before by [13]. On the day preceding last testing (for example twentieth day of dosing), every mouse is set on the way to the finish of an open arm, confronting towards the focal stage. TL is characterized as the time it took for mice to move from open arm into the enclosed arms. Transfer Latency is recorded for every mouse. Animals were reanalyzed 24 h after the preliminary training to test retention of memory.

**4.3 Biochemical estimations groups**

All groups of mice later than behavioral study were sacrificed and then cerebrum and blood were taken out for biochemical estimations of cholinesterase and nitrite from brain, and corticosterone from plasma. For these estimations different groups were utilized. These are as per the following:

**4.3.1. Determination of corticosterone level**

On the twenty first day after given all dose of drugs with stress and non stress procedures gathered the blood from retro-orbital plexus method, and plasma was isolated by centrifugation at 2500 rpm. By using Bartos and Perez, 1979 [14] method corticosterone estimation from plasma was performed. Using corticosterone acetate with standard curve equation(Y=0.008X+0.046)the standard curve was plotted. Different Groups for plasma corticosterone levels:

Groups XI: For 21 progressive days Metyrapone (10 mg/kg) was given. On the last day, after 35 min of drug dosing, the mice were yielded and evaluated the plasma corticosterone levels.

Groups XII: For 21 progressive days Metyrapone (10 mg/kg) was given alongside the chronic stress method, the mice were sacrificed on the last day, after 35 min of drugs dosing and evaluated the plasma corticosterone levels.

**4.3.2. Determination of brain nitrite level**

By utilizing the strategy of Green et al., 1982 brain nitrite was estimated [15]. The absorbance was perused at 546 nm. By utilizing sodium nitrite with equation (Y=0.0035X+0.1056) standard curve was plotted

For estimation of brain nitrite levels different groups are as fowling:

Group XIII: For 21 progressive days l-arginine (100 mg/kg) was regulated. On the last day, after given drugs and after 35 min the mice were decapitation and nitrite level was estimated by using collected brain.

Group XIV: For 21 progressive days l-arginine (100 mg/kg) was regulated alongside chronic stress technique. On the last day, 35 min after the last portion, the mice were yielded and estimation of nitrite levels was done by using brain homogenate.

Group XV and XVI: For 20 progressive days Alcoholic concentrate of Mexican poppy (500mg/kg i.p) and Normal saline independently were infused to these groups, on the last dayafter all drugs was given then after 35 minute 7-Nitroindazole (20 mg/kg) was infused. The mice were sacrificed 35 min after given of the 7-Nitroindazole.

Groups XVII and XVIII: alcoholic concentrate of Mexican poppy (500mg/kg i.p) and Normal saline independently were infused to various groups for 20 progressive days alongside the stress method, on the last day, after all drugs was given then after 35minute aminoguanidine (50 mg/kg) was infused, and the mice were killed35 min after given of amino-guanidine .

### 4.3.3. Determination of Brain Acetyl cholinesterase

By utilizing the strategy for Ellman et al., 1961 after sacrificing animal brain was removed and using homogenizer homogenized the brain in 0.1M phosphate buffer with pH 7.2. Brain acetyl cholinesterase was performing based on colorimetric method by using acetylcholine iodide as a substrate. [16].

### 4.4. Statistical Analysis

Observation is spoken to as Mean ± Standard Error. The examination of information was finished by utilizing one way ANOVA. The degree of Freedom was checked by Tukey's Test.

## 5. RESULTS

### 5.1. Impact of alcoholic concentrate of Mexican poppy on Transfer Latenciesin an Elevated plus Maze of scopolamine induced impairment in mice

Exposure of mice to chronic Stress method was responsible for weaken memory as appeared by a huge increment in TL. Scopolamine induced group showed a significant $F(4, 25) = 39.183$; $p<0.001$ (non stressed mice) and $F(4,25) = 49.730$ ; $p<0.001$ (chronic stressed mice) increase in transfer latencies and retention of memory when compared to normal saline, standard drug and extract group of Mexican poppy for both non stress & stress groups respectively, Whereas piracetam (200 mg/kg i.p.) standard drug group when compared to Alcoholic concentrate of Mexican poppy (500 mg/kg, i.p.) showed significantly ($p<0.01$)i.e decrease in transfer latencies and retention of memory for both non stressed & stressed mice as shown in Table 1.

**Table 1:** impact ofalcoholic extract of Mexican poppy on TL using EPM for 21days

| Drugs Dosing | TL on 21st daynonstressed mice | TL on 21st dayStressed mice |
|---|---|---|
| Normal saline 10ml | 29.66 ± 2.61 | 38.66 ± 2.89 |
| Standard drug Piracetam 200mg | 8.5 ± 0.99* | 13.33 ± 1.33# |
| Scopolamine 0.4mg | 35.16 ± 3.27* | 63.5 ± 3.09# |
| Alcoholic extract of Mexican poppy 500mg | 13 ± 1.93* | 33.66 ± 1.49# |
| Alcoholic extract of Mexican poppy 500mg + Scopolamine 0.4mg | 27.16 ± 2.19 | 38.5 ± 2.93 |

{Observations are articulated as Mean ± SEM; difference from control group unstressed mice is significant and shown as*= ($p<0.01$); difference from control group stressed mice is significant and shown as#= ($p<0.01$)}

### 5.2. Impact of alcoholic concentrate of Mexican poppy on TRC in Hebb Williams Maze of scopolamine induced impairment in mice

Exposure of mice to chronic Stress method was responsible for weaken cognition as appeared by a huge increment in Discrimination Index. Scopolamine induced group showed a significant $F(4, 25) = 40.694$; $p<0.0001$; (non stressed mice) and $F(4, 25) = 50.078$; $p< 0.0001$;(chronic stressed mice) increase in Discrimination Index and retention of memory when compared to normal saline, standard drug and extract group of Mexican poppy for both non stress and stress groups respectively, Whereas piracetam (200 mg/kg i.p.) standard drug group when compared to Alcoholic concentrate of Mexican poppy (500 mg/kg, i.p.) showed significantly ($p<0.01$) i.e decrease in Discrimination Index and retention of memory for both non stressed & stressed mice as shown in Table 1

**Table 2:** impact of alcoholic extract of Mexican poppy on TRC using HWM for 21 days

| Drugs Dosing | TRCon 21st dayNon stressed mice | TRCon21st dayStressed mice |
|---|---|---|
| Normal saline 10ml | 88 ± 2.95 | 109.83 ± 20.83 |
| Standard drug Piracetam 10mg | 24.5 ± 4.06* | 48.33 ± 11.85# |
| Scopolamine 0.4mg | 114.83 ± 6.99* | 114.66 ± 3.57# |
| Alcoholic extract of Mexican poppy 500mg | 58 ± 8.12* | 65 ± 13.79# |
| Alcoholic extract of Mexican poppy 500mg + Scopolamine 0.4 | 86 ± 1.98 | 83.66 ± 4.58 |

(Observations are articulated as Mean ± SEM. difference from control group unstressed mice is significant and shown as*= ($p<0.01$); difference from control group stressed mice is significant and shown as #= ($p<0.01$))

### 5.3. Impact of Alcoholic extract of Mexican poppy on brain acetylcholinestrase

Chronic Stresshas no significant alteration in brain acetylcholinesterase activity in compression to control group of nonstressed mice. In control group AchE was very low (5.15 ± 0.11) in unstressed mice. But negative control group of Scopolamine (0.4 mg/kg) has shown a significant increase in brain AChE activity (11.22 ± 0.30*). Mexican poppy (500 mg/kg) and piracetam (200 mg/kg) significantlyp <0.0001lowered the brain AChE activity in both unstressed & stressed mice. Mexican poppy (500 mg/kg) shows anti acetylcholinestrase effect and have nootropic activity (Table 3).

**Table 3:** impact of Alcoholic extract of Mexican poppy on brain acetylcholinestraselevels of nonstressed& stressed mice for 21 days

| Drug Dosing | Brain acetylcholinestrase(m mol/min/mgt issue) Non stressed animal | Brain acetylcholinestrase(m mol/min/mg tissue) Stressed animal |
|---|---|---|
| Normal saline 10ml | 5.15 ± 0.11 | 4.75 ± 0.23 |
| Standard drug Piracetam 200mg | 2.57 ± 0.22* | 2.23 ± 0.25# |
| Scopolamine 0.4mg | 11.22 ± 0.30* | 9.15 ± 0.76# |
| Alcoholic extract of Mexican poppy 500mg | 2.73 ± 0.18* | 2.68 ± 0.79# |
| Alcoholic extract of Mexican poppy 500mg + Scopolamine 0.4mg | 6.04 ± 0.37 | 4.63 ± 0.21 |

{Observation are articulated as Mean ± SEM. difference from control group unstressed mice is significant and shown as *= (p<0.01); difference from control group stressed mice is significant and shown as #= (p<0.01)}

### 5.4. Effects of Alcoholic extract of Mexican poppy on Brain Nitrite Levels

Chronic stress showed a sharp increase in brain nitrite levels. Alcoholic extract of Mexican poppy (500 mg/kg), Aminoguanidine &7-Nitroindazole significantly (p<0.01) lowered the brain nitrite in both chronically stressed and nonstressed mice. In nonstressed mice 7-Nitroindazole and in stressed mice aminoguanidine, symbiotically lowered brain nitrite with Mexican poppy extract (Table 4).

**Table 4:** Effect of Alcoholic extract of Mexican poppy on brain nitrite levels of nonstressed & chronically stressed mice

| Drug Dosing for 21 days/kg | Brain nitrite levels (µg/ml) Nonstressedanimal | Brain nitrite levels (µg/ml) Stressed animal |
|---|---|---|
| Normal saline 10ml | 20.59 ± 0.26 | 27.99 ± 0.75 |
| l-arginine 10mg | 27.48 ± 0.50* | 37.01 ± 0.33# |
| Alcoholic extract of Mexican poppy 500mg | 12.51 ± 0.49* | 14.4 ± 0.46# |
| Normal saline + 7-NI (unstressed mice) | 14.21 ± 0.35* | ------ |
| Alcoholic extract of Mexican poppy + 7-NI (U) 500mg +20mg | 8.61 ± 0.81* | ------ |
| | | |
| Normal saline + aminoguanidine (stressed mice)10ml +50mg | ------ | 13.55 ± 0.31# |
| Alcoholic extract of Mexican poppy + AG (S)500mg+50mg | ------ | 9.98 ± 0.92# |
| | | |

(Observations are articulated as Mean ± SEM. difference from control group unstressed mice is significant and shown as*= (p<0.01); difference from control group stressed mice is significant and shown as #= (p<0.01)

### 5.5. Alcoholic extract of Mexican poppy Effects on blood Corticosterone Levels

Chronic Stress raised corticosterone levels in blood and Metyrapone (10 mg/kg) extensively lowered plasma corticosterone in both chronically stressed & non stressed mice. Mexican poppy (500 mg/kg) not indicating any major impact on plasma corticosterone in non stressed mice anyway Mexican poppy (500 mg/kg) extensively brought down the plasma corticosterone in chronically stressed mice (Table 5).

**Table** 5 Effect of Alcoholic extract of Mexicana poppy on plasma corticosterone levels of non stressed& stressed mice for 21 days

| Drug Dosing | Cortico. level (µg/dl) Non stressed animals | Cortico. level (µg/dl) Stressed animals |
|---|---|---|
| Normal saline 10ml | 11.87 ± 0.28 | 21.75 ± 0.35 |
| Metyrapone 10mg | 6.52 ± 0.23* | 8.51 ± 0.26# |
| Alcoholic extract of Mexican poppy 500mg | 9.91 ± 0.32* | 11.02 ± 0.25# |

{Observations are articulated as Mean ± SEM. difference from control group unstressed mice is significant and shown as *= (p<0.01); difference from control group stressed mice is significant and shown as #= (p<0.01)}

## 6. Discussion

Current investigation of Alcoholic concentrates of Mexican poppy (500 mg/kg, i.p.) given regularly for 21days established remarkable nootropic action in unstressed and stressed mice. The way that the Alcoholic concentrate of Mexican poppy crosses the blood-brain barrier is supported by some other research. It is very much clear that UCMS produces similar types of physiological changes in mice as that of human beings. Several studies show that chronic stress increases cognitive impairment which result in depression, anxiety, impairment of learning and memory [17]. Hence it can be understood that there is a strong relation between AchE activity, cholinergic system and memory. The treatments which upgrade cholinergic capacity can be utilized for the recovery of cognitive debilitation. Acetylcholinesterage inhibitory action of Alcoholic concentrate of Mexican poppy (500 mg/kg, i.p.) turned around scopolamine-induced cognitive impairment and diminished brain cholinesterase levels in unstressed and stressed mice. Subsequently, the plausible component of nootropic activity of Alcoholic concentrate of Mexican poppy might be, the decline in brain AChE levels. The use Piracetam (200mg/kg, i.p) standard drug significantly lowered the AchE level indicating the stimulatory action of this drug on cholinergic system. Corticosterone levels were seen as expanded in stressed mice because stress is responsible for the release of CRH, ACTH, and cortison. Alcoholic concentrate of Mexican poppy (500 mg/kg, i.p.) and metyrapone (10mg/kg i.p) decreased level of plasma corticosterone in stressed mice. Along these lines, the memory-upgrading impact of Alcoholic concentrate of Mexican poppy in stressed mice may likewise be credited to its cancer prevention agent property. Alcoholic concentrate of Mexican poppy essentially diminished the brain nitrite levels in both non stressed and chronically stressed mice. In nitric oxide method nitric oxide released from sodium nitroprusside which reacted with oxygen and form nitrite ion and Griess reagent can estimate this ion presance. 7-Nitroindazole which is nNOS inhibitor synergistically diminished cerebrum nitrite levels in non stressed mice when directed with Mexican poppy. This shows the memory-improving action of Mexican poppy in unstressed mice by inhibiting nitrite formation by competing with the oxygen atom to react with nitric oxide. Antioxidant activity of alcoholic extract of Mexicana poppy provided mechanism by inhibiting of neuronal NOS. similarly alcoholic concentrate of Mexican poppy reduced brain nitrite levels in non stressed animals as well as in stressed mice by inhibiting iNO S& n NOS.

## 7. Conclusions

Alcoholic extract of Mexican poppy has memory-improving action in non stressed mice by diminishing AChE activity and inhibition of nitric oxide release by inhibiting neuronal NOS. Then again, Alcoholic extract of Mexican poppy demonstrated memory-improving activity in chronically stressed mice by diminishing brain AChE action and inhibiting of inducible NOS and furthermore by diminishing the raised degrees of plasma corticosterone. In this manner, the Alcoholic concentrate of Mexican poppy must be investigated further for its potential as nootropic agent.

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